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EFFECT OF CALCIUM-DEFICIENT ROUGHAGES UPON MILK YIELD AND BONE STRENGTH IN CATTLE

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INTRODUCTION

Dairy cows depend upon roughages as the principal natural sources of calcium, and upon grains and milling by-products for most of the phosphorus. Grasses provide from one-fifth to one-third as much calcium, and about three-fifths as much phosphorus as do the legumes grown under the same conditions. The calcium content of the forages grown on low-lime soils is lower than that of the same kinds of plants on more fertile soils. Lactating cows dependent on grass forages grown on low-lime soils often deplete their reserves of calcium to such an extent that skeletal strength and yield of milk are affected.

STATEMENT OF PROBLEM

The Jersey cows in the Florida Agricultural Experiment Station dairy herd were dependent upon pasture grasses, and corn or sorghum silages, as the main sources of roughages. Since December, 1920, alfalfa meal was included as one-ninth to one-eighth of the concentrates mentioned in the herd records and annual reports of the station. One cow—No. 18—received alfalfa meal prior to that time, while she was on Register of Merit test.

All rations mentioned during this time contained an adequate proportion of protein, total digestible nutrients and phosphorus for cows of their weight and milk production. Under the conditions of feeding just mentioned, these Jerseys had an average milk yield of approximately 4,000 pounds per year. A number of them suffered broken bones—hips, ribs and even a pelvis—under this general feeding practice.

The rations were changed in January, 1929, by the addition of two per cent of bonemeal to the mixed concentrates, based upon a study of the conditions noted. Bonemeal was chosen in preference to a local supply of calcium carbonate because the former was known to be free from fluorine and other undesirable substances. This proportion of mineral supplement

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was assumed to supply sufficient calcium to provide for maintenance, a reasonable milk production, and to allow a surplus with which the cows could restore the body reserves.

REVIEW OF LITERATURE

Eckles (2) observed that cows on phosphorus-deficient rations produced much less milk than when the lacking mineral was supplied. Theiler (7) and Tuff (8) reported similar conditions among cattle in parts of the Union of South Africa and of Norway. Fingerling (3) obtained similar results with milk goats on rations the basal portion of which was deficient in calcium. Meigs and Woodward (5) concluded that cows produced appreciably more milk after having been placed in good mineral storage during the preceding dry period.

Kellner (4) mentioned that dairy rations are deficient in calcium more frequently than they are in phosphorus. Under these conditions, cows withdraw mineral matter from the bones in order to maintain milk production. As this impoverishment progresses, the milk yield is decreased.

Nessler (6) found that the bones of a cow affected with *Knochenbruchigkeit* (bone-fragility) contained less calcium and phosphorus, were lighter in weight, and had thinner shaft walls than had those of a healthy cow. The spongy bones—vertebrae, pelvis and joints—were affected more than the shaft bones of the legs. This depletion of calcium and phosphorus in the skeleton occurred when the cattle received feeds inadequate in the bone-forming mineral elements.

PLAN OF INVESTIGATION

Typical rations used with the dairy herd were assembled from the herd records and annual station reports, and a study made of them. The rations were changed slightly in January, 1929, by adding two per cent of finely ground feeding bonemeal to the concentrates. Some of the higher producing cows were given a daily allowance of about five pounds of alfalfa hay. Some changes were made in the proportion of concentrates based on local market prices of nutrients, and upon availability of these feeds.

Twelve Jersey cows in the herd had completed lactations on the unsupplemented rations, and also upon the supplemented rations after January, 1929. Their lactation records were assembled by 10-day periods, computed to maximum age basis, using the factors of Clark (1) for Jersey cows milked twice daily not under official test conditions. An average lactation curve was computed, weighted on the "per cow" basis.

Cow No. 59 broke her pelvis late in December, 1928. Following the autopsy, breaking strengths were determined upon her femurs and humeri, using an electrically driven Riehle Brothers testing machine, and applying the weight slowly from above in the middle of 7- and 5-inch spans of the

shafts. After bonemeal had been added to the rations, leg bones were obtained from additional Jersey cows as they were eliminated from the station herd, and bone strengths determined similarly, using a 6-inch span. Similar data were obtained from the leg bones of three range cows, six Aberdeen Angus cows and 24 three-year old steers. The steers had free access to bonemeal during one year prior to slaughter, while the Angus cows received a limited grain allowance which contained one per cent of bonemeal. Both the steers and Angus cows received pasture grasses and silages grown on the same types of soil as that fed to the Jerseys.

PRESENTATION OF DATA

The data presented herein were accumulated under the conditions of management prevailing over a period of years in the station dairy herd generally, rather than from an investigation planned in advance with a limited number of selected cows. Detailed search of the herd records and experiment station reports showed that pasture grasses (largely Bahia, centipede and Bermuda grasses), corn, cane or sorghum silages were the principal roughages in use. Summation of published data from seven early feeding trials between 1908 and 1917 showed that Jersey cows with an average weight of 711 pounds and a 15 pound milk yield daily, received 22¼ pounds of silage. Their concentrates were mainly one part of cottonseed meal (36% protein) with 2 to 4.4 parts of wheat bran. Other concentrates fed at times included cocoanut meal, wheat middlings, velvet beans in the pods or ground into feed meal. One test cow ration consisted of 300 wheat bran, 200 velvet bean feed meal, 100 cornmeal and 80 parts of peanut meal (grade not stated). Alfalfa meal was mentioned first in the ration of Cow No. 18 on Register of Merit test, and again in December, 1920, when it became one-ninth to one-eighth of the regular concentrate mixtures fed. During two feeding trials of 123 and 84 days, 4 and 6 cows respectively received not in excess of four pounds of alfalfa hay daily. Mineral supplements, except common salt, were not used until shortly prior to 1929, and then for but a limited time.

The concentrate mixtures used more recently in connection with the roughages mentioned are listed in table 1. Cost and availability of oats, corn gluten feed and peanut meal (44% protein) caused them to be replaced with locally grown velvet bean feed meal, higher grade cottonseed meal and by changing the proportions of the feeds used.

Calcium and phosphorus contents of the several typical rations were calculated, using average analyses of purchased concentrates, and analyses of the locally-grown feeds. All of these rations provided an adequate supply of protein and total digestible nutrients, and exceeded the requirements given by Kellner (4) and Wellman (9) for phosphorus. The supply of calcium was noticeably inadequate.

TABLE 1

Concentrates used with silages and grass pasture in feeding the Florida Station dairy herd from 1922 to 1933

YEAR	1922	1923	1924 AND 1925	1926	1929 TO 1933
Ration	Ration 1	Ration 2	Ration 3	Ration 4	Ration 5
Wheat bran	100	100	100	200	400
Cornmeal	100	100	100	300	300
Ground oats	75	75	100	300	—
Alfalfa meal	50	50	50	—	—
Peanut meal	50	—	—	100	—
Cottonseed meal, 36%	50	100	100	100	—
Cottonseed meal, 41%	—	—	—	—	100
Corn gluten feed	—	—	—	100	—
Dried beet pulp	—	—	—	100	200
Linseed oilmeal	—	—	—	100	100
Velvet bean feed meal	—	—	—	—	200
Common salt	—	—	—	—	13
Bonemeal	—	—	—	—	26

The concentrates in general use since January, 1929, containing two per cent of bonemeal, provided calcium sufficient to meet the requirements for maintenance, a more liberal milk yield, and to allow mineral reserves to be restored to the skeleton. No cow in milk in the station herd has had a broken bone since that time, and the milk yield has increased, as will be shown. A comparison of the amounts of calcium and phosphorus contained in these several rations is shown in table 2.

Lactation records were assembled and tabulated for the 12 Jersey cows that had completed lactations both on the earlier low-calcium rations with the concentrates listed in table 1, and on the rations containing bonemeal. A total of 44 lactations were included in the low-calcium group. The average *actual* yield per lactation, weighted on the "per cow" basis, was 3,980 pounds of milk, or an average of 13.38 pounds daily during these 44 lactations of 400 days or less. These same 12 cows completed 22 lactations on the high-calcium rations, averaging 6,425 pounds of milk, or 17.55 pounds daily. The earlier group of lactations averaged 297 days in length, in contrast to 366 days for the latter group.

There was a marked tendency for these cows to attain a higher maximum daily milk yield, and to decline in milk flow less rapidly when the low-calcium rations had been supplemented with bonemeal.

Since these lactations were begun at varying ages, they have been computed to a uniform maximum age basis, using the factors obtained by Clark (1) with Jersey cows milked twice daily not on official test. The lactation curves so computed, are shown in figure 1. These milk records

TABLE 2

Comparison of the nutrients required by a typical cow in the station dairy herd and of the nutrients provided by rations in use between 1908 and 1933

	RATIONS		NUTRIENTS PROVIDED			
	Corn silage	Concentrates	Digestible crude protein	Total digestible nutrients	Calcium (Ca)	Phosphorus (P)
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Requirements—						
725 lb. cow yielding 15 lbs. of 5% milk*	.....	.....	1.5050	11.4756	0.0800–.1059 .0789***	0.0394–.0458** .0323***
Rations in use—						
1908–1917	22.25	12.0	2.3178	11.6653	.0306	.1705
1922–1925	22.25	11.0	1.907	11.638	.0448	.1973
1928	22.25	10.5	1.8733	11.6600	.0408	.1535
1929–1933	22.25	11.0	1.6811	11.6075	.0776	.1032

*—The average requirements are based on weights and milk records of cows in feeding trials conducted between 1908 and 1917 at this station.

**—Calcium and phosphorus requirements calculated according to Wellman's (9) requirements.

***—Calcium and phosphorus requirements calculated according to Kellner's (4) requirements.

were assembled by 30-day periods, and the relative rates of milk yields calculated in relation to the milk yield on the unsupplemented rations. This

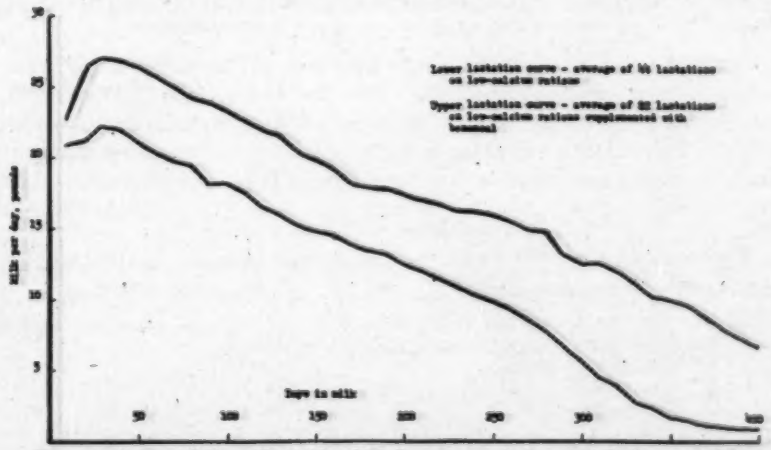

FIG. 1. Average daily milk yields of 12 Jersey cows before and during use of bonemeal as a supplement to low-calcium rations.

comparison of monthly milk yields on the low-calcium and on the supplemented rations is shown in table 3.

TABLE 3

Persistence of milk production of 12 Jersey cows as affected by addition of bonemeal as a supplement to low-calcium rations

MONTH	PRODUCTION ON LOW-CALCIUM RATIONS		PRODUCTION ON SUPPLEMENTED RATIONS	
	Milk yield	Rate of production*	Milk yield	Rate of base production*
	pounds	*per cent*	*pounds*	*per cent*
1	644.3	13.84	765.1	16.43
2	636.2	13.66	789.9	16.96
3	572.1	12.29	732.1	15.72
4	525.5	11.29	681.3	14.63
5	464.0	9.96	622.2	13.36
6	420.6	9.03	553.2	11.88
7	378.2	8.12	525.3	11.28
8	328.2	7.05	493.7	10.60
9	281.2	6.04	464.1	9.97
10	201.9	4.34	406.7	8.73
11	112.6	2.42	359.0	7.71
12	55.4	1.19	295.4	6.34
13	27.6	.59	237.7	5.11
10 days	8.4	.18	66.2	1.42
Total	4,656.2	100.00	6,991.9	150.16

*—The total production in the first lactation curve (4,656.2 pounds of milk) is the base figure used in computing the rate of production. These lactation curves are weighted on the "per cow" basis, computed to the maximum age by using the factors of Clark (1), so as to place individual lactations upon a comparable basis.

Over fifty per cent more milk was produced per lactation of 400 days or less on the supplemented rations. These 12 cows yielded 31.17 per cent more milk per day for each day in milk (under 400 days) during these lactations. This latter point takes into consideration the fact that the cows tended to milk over longer lactation periods on the supplemented rations.

SKELETAL STUDIES

Five cows in a herd of 34 on the low calcium diet had suffered one or more broken bones, as follows:

No. 59—pelvis broken in three places.
No. 120—both hips and right 12th rib broken.
No. 223—right 13th rib.
No. 225—left hip and last four ribs on right side.
No. 229—left hip.

Even a 10-year old bull—Florida's Majesty 153431—was slaughtered in 1927 because of a broken hip that affected his usefulness. This large pro-

portion of animals with broken bones called attention to the extremity of the condition caused by the low-calcium rations. Cow No. 59 went down with a broken pelvis during late December, 1928. Upon autopsy, the femurs and humeri were obtained, and average breaking strengths of only 335 pounds were recorded. In these determinations, 7- and 5-inch spans respectively were used, but in all subsequent studies of bone strengths, a standard 6-inch span was adopted. As more cows were eliminated from the herd, the long bones of the legs were obtained, and their strengths determined.

It is of interest to note that the femurs and humeri of four Jersey cows receiving the supplemented ration for the last 19 to 27 months, averaging over 10 times as strong as the corresponding bones taken from Cow No. 59. In every instance the femurs and humeri exceeded the cannon bones in average strength. Noting this relationship, it was possible to use strengths of cannon bones as indices of the state of mineral storage of the cows.

The strengths of No. 59's leg bones represent an extreme stage of depletion of mineral reserves in the skeleton. This cow had been a persistent producer, averaging 6,338.6 pounds of milk in her 11 lactations, and in the last lactation produced 8,151.9 pounds of milk in 531 days. That this cow is not an isolated instance is suggested by the proportion of cows in the station herd at that time that had broken one or more bones.

The strengths of cannon bones from 24 three-year old steers represent a medium stage of mineral storage in the skeletal tissues. Three mature (dry) range cows for which bone strengths are presented, were in a lower stage of storage. On the other hand, the bone strengths of the dairy cows that had received the supplemented rations indicate that it may be possible for cows to attain a stage of excellent mineral storage, available for subsequent lactations. A summary of these bone strengths, as determined on selected bones taken from cattle under the various conditions outlined, are presented in table 4.

DISCUSSION OF RESULTS

The cows considered in this study received rations that were adequate, considered from the usual feeding standards. This was evidenced by the fact that many of the cows were extremely fat, and yet were not yielding milk in proportion to the intake of digestible nutrients—in many instances they had one pound or more of grain for each two pounds of milk produced. The quality of protein was probably satisfactory, since it was derived from six plant sources—maize, wheat, flax, velvet beans, cotton and sugar beets—in addition to the variety of pasture grasses. The supply of phosphorus from the concentrates was in excess of the requirements. The calcium intake in the average ration was extremely low, even for cows with the level of milk production of Jerseys in commercial dairies.

The depression of milk production was less marked than has been observed in long-continued phosphorus deficiency (2).

TABLE 4

*Average breaking strength of 215 leg bones from Florida cattle under different feeding conditions**

CATTLE	TYPE OF RATION	AVERAGE BREAKING STRENGTH OF BONES FROM LEFT AND RIGHT LEGS						
		Humerus	Femur	Radius and ulna	Tibia and fibula	Fore cannon	Rear cannon	Average
				No bonemeal available				
Jersey		*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Cow No. 59	Dairy ration, unsupplemented	330[a]	340[b]	.....	.....	.....	.....	335
Range Cows								
No. 1	Range on sand and muck lands	.....	.....	.....	.....	1,530	1,998	1,764
No. 2	Range on sandy lands	2,240	2,405	1,923	2,602	1,883	2,155	2,201
No. 3	Range; peanut hay in winter	.....	.....	.....	.....	2,120	2,380	2,250
				Two per cent of bonemeal in concentrates				
Guernsey								
Cow No. 297	Dairy ration; bonemeal 1 mo.	.....	.....	.....	.....	3,470	3,440	3,455
Dutch Belted Cow	" " ; bonemeal 29 mo.	3,535	3,645	3,710	3,240	3,255	3,868	3,542
Jersey Cows								
No. 81, 120, 195, 225	" " ; bonemeal 19–27 mo.	3,244	3,636**	2,848	3,299	2,228	3,302	3,037
No. 177, 188, 218	" " ; bonemeal 13–23 mo.	.....	.....	.....	.....	2,700	3,788	3,244
				One per cent of bonemeal in concentrates				
Aberdeen Angus								
6 cows	Grass, silage, concentrates	.....	.....	.....	.....	2,689	3,370	3,030
				Access to bonemeal, ad libitum				
Steers								
8 grade Angus	Grass pasture, with peanut hay in winter	.....	.....	.....	.....	2,059	3,059	2,559
16 native and grade Herefords	Grass pasture, with peanut hay in winter	.....	.....	.....	.....	2,426	3,184	2,805

*—Breaking strengths were determined by using a 6-inch span with weight applied slowly in the middle from above, except (a) a 5-inch span, and (b) a 7-inch span with Cow No. 59.

**—One femur of Cow No. 195 not available.

Under this condition of inadequate calcium intake, not only were milk yields less than expected, but mineral reserves were depleted to the point of skeletal fragility. A significant proportion of the cows had broken ribs or hips. Since the correction of the calcium deficiency by the addition of bonemeal to the rations, the cows have suffered no broken bones in the next four years, mineral reserves (as measured by bone strengths of cows slaughtered) have been restored, and milk yields have been attained commensurate with inheritance and the organic nutrient intake of the same cows.

STATISTICAL ANALYSIS

Study of the records for possible contributing factors arising from management of the cows disclosed that the dry periods prior to lactation averaged 80 days in length preceding use of bonemeal, as against 86 days while bonemeal was available. This excludes the 12 lactations after the first parturition of each cow in the former period, and one instance of difficult conception among the 22 lactations in the latter period. The average date of conception was at 105 days after parturition in the former interval, as against 169 days (*including* five cases of difficult conception of 300 days or over) in the latter period. In addition, cystic ovaries were encountered in two old cows, and four failed to conceive. The observation of Dr. C. H. Eckles in Minnesota Station Bulletin 258, was borne out in that the older cows conceived less readily.

From statistical analysis of differences in rate of decline between milk production on the low-calcium rations, and that on the supplemented rations, it was found that the standard error of difference, divided into the difference is 1.73, or that the probabilities are 9 in 10 times that the differences are not due to chance. In other words, these differences are due to inherent differences in the rate of production rather than to chance. This took into consideration the entire lactation curves from the second to thirteenth months inclusive.

The entire lactations include the period immediately after calving, during which the mineral matter stored in the skeleton in the dry period was available. After this available supply is depleted by lactation, the cows become dependent upon the limited amounts in the feed, and milk secretion is checked. When the curves for the 7th to 13th months were analyzed similarly, the factor 72.17 was found in place of 1.73. In other words, it is highly significant that these differences are inherent, rather than due to chance.

SUMMARY AND CONCLUSIONS

Typical rations used in feeding the Jersey cows at the Florida Agricultural Experiment Station during a period of years supplied an excess of protein, energy and phosphorus, but were low in content of calcium. Addition of bonemeal as two per cent of the concentrates was sufficient to render the calcium level adequate for a commercial dairy herd.

This increase in calcium level in the rations allowed 12 Jersey cows to attain an increase of four pounds of milk per day in subsequent lactations, and to be more persistent producers throughout longer lactation periods. At the same time, these cows attained a stage of mineral storage in the skeletal tissues such that the leg bones from nine of them had average breaking strengths in excess of 3,000 pounds. On the other hand, in absence of the calcium supplement, several of these same cows previously had withdrawn mineral reserves from the skeleton to such an extent that an unusual proportion of them had suffered broken hips and ribs.

The possibility of corn silage, grown on low-calcium soils, as a source of roughage for use in studies of calcium metabolism is suggested.

ACKNOWLEDGMENTS

The lactation periods prior to May 15, 1928, were accumulated under the direction of Professor John M. Scott, formerly Animal Industrialist and vice-director of the Florida Agricultural Experiment Station. Alex R. Mathers assisted in assembling and tabulating the milk records. Professor C. H. Willoughby allowed us to obtain leg bones from Aberdeen Angus cows for use in this study. Professor Charles C. Brown of the Civil Engineering Department made available such equipment as was used in bone strength determinations. Bradford Knapp, Jr., and Dr. L. W. Gaddum assisted with the statistical analyses, testing the significance of the data presented herein.

REFERENCES

1. Clark, R. S. The correlation between changes in age and milk production of dairy cows under other than official testing conditions. Journal of Dairy Science 7: 547–554. 1924.
2. Eckles, C. H. Some phases of the phosphorus deficiency problem in the nutrition of dairy cattle. Presented before Amer. Dairy Sci. Assoc., Washington, D. C., June 26, 1929.
3. Fingerling, G. Beitrage zur Verwertung von Kalk- und Phosphorsäureverbindung durch den tierischen Organismus. Landw. Vers. Stat. 75: 1–52. 1911.
4. Kellner, D. Die Ernahrung der landwirtschaftlichen Nutztiere. Paul Parey, Berlin. 10th edit. 1924. Pages 618–619.
5. Meigs, E. B. and Woodward, T. E. The influence of calcium and phosphorus in the feed on the milk yield of dairy cows. U. S. Dept. Agr. Bul. 945: 1–28. 1921. Also Journal of Dairy Science 4: 185–217. 1921.
6. Nessler, J. II. Untersuchungen der Knochen von knochenbruchigkeit Rindvieh. Landw. Vers. Stat. 16: 187–196. 1873.
7. Theiler, Sir A., Green, H. H. and du Toit, P. J. Phosphorus in the livestock industry. Jour. Dept. Agr. (Union of S. Africa) 8: 460–504. 1924.
8. Tuff, P. Osteomalacia and its occurrence in cattle in Norway. Jour. Comp. Path. and Ther. 36: 143–155. 1923. Also World's Dairy Congr. II: 1494–1501. 1923.
9. Wellman, O. Futterungsversuche uber die Bedeutung des Mineralstoffwechsels, insbesondere der Erdalkalien und Phosphorsaure. Biologia Generalis, 8: 387–396. 1932.

THE DETECTION OF THE ESCHERICHIA–AEROBACTER GROUP IN BUTTER

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A plentiful supply of pure water is a valuable asset in the manufacture of butter of good quality; and therefore the employment by the dairy bacteriologist of a simple routine method for the detection of the Escherichia-Aerobacter group in butter is useful as a guide to the character of the water used for washing the butter during the process of manufacture.

Hammer and Yale (1) have shown that the growth of the Aerobacter species is more injurious to the keeping quality of the butter than the growth of the Escherichia species; that the Aerobacter species when butter was held at 18° C. regularly developed off odors and flavors in either salted or unsalted butter.

An old method for the detection of the Escherichia-Aerobacter group was the use of litmus lactose bile medium, but it is known that this medium favors the growth of the Escherichia group; and in "Standard methods of Water Analysis, 1925," it is recommended that a standard lactose broth medium be used. If there is formation of 10 per cent or more of gas in a standard lactose broth fermentation tube within 24 hours at 37° C., it is recommended to make one or more Endo- or Eosin-Methylene-blue plates from the tube which shows gas formation from the smallest amount of water tested, and to note the type of growth when the Petri dishes are incubated at 37° C. for 24 hours. Absence of gas formation after 48 hours' incubation constitutes a negative test, and there is a note—"an arbitrary limit of 48 hours' observation doubtless excludes from consideration occasional members of the Coli-Aerogenes group which form gas very slowly, but for the purpose of a standard test the exclusion of these occasional slow gas-forming organisms is considered immaterial."

The presence and detection of these slow gas-forming organisms is of importance to the dairy bacteriologist, since there are members of the subgenus Aerobacter whose optimum temperature is about 25° C. and which give only scanty growth when growth at 37° C. on standard lactose nutrient agar, and show little or no gas formation when grown in standard lactose broth solution and held at 37° C. for a period of 24 to 48 hours, while they produce a marked amount of gas in the fermentation tube when

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held at 21° C. to 25° C. for 24 hours. The detection of the Aerobacter Group in butter is of importance, since these originate from soil, grains or feeds, and if found in butter their presence shows contamination of the water used at the creamery, or of the cream after pasteurization.

Various workers have found that lactose peptone broth solution is not a reliable enrichment medium for the Escherichia-Aerobacter group. This has led to the use of dyes having a selective bactericidal action and we have the Gentian violet lactose peptone bile broth of Kessler and Swenarton (2), the Trypaflavin lactose broth of Klimmer and coworkers (3), and the Brilliant green bile broth of Muer and Harris (4). Solid media for direct estimation such as Eosin methylene-blue agar have been used for direct count; but for routine work, where the percentage of positive results is not high, as in the bacteriological analysis of butter, a simpler method of detecting the presence of the Escherichia-Aerobacter group is desirable.

In the routine examination of butter in our laboratory it is usual to add one gram of the emulsified butter to 9 cc. of warm water, and, after mixing thoroughly, to pipette 1 cc. of the dilution into each of two fermentation tubes containing standard lactose broth solution and two tubes of sterile litmus milk. One of each pair is inoculated at 21° C. and the other two at 37° C. Results obtained over a period of several years have shown that at times gas will be produced at 21° C. in both fermentation tube and litmus milk when there is no evident gas formation at 37° C., and streaking on Eosin-Methylene-blue plates shows the presence of members of the subgenus Aerobacter. It has been noticed that coagulation of the milk with acid and gas formation occurs at times at both 21° C. and 37° C. when there has been no evident gas formation in the fermentation tube, and when a portion of the milk serum was streaked on an Eosin-Methylene-blue plate, a typical Escherichia or Aerobacter growth was obtained.

It is recommended, therefore, in the routine examination of butter to follow the usual procedure of placing the sample bottle containing the butter to be tested, in a water bath held at 40° C. to 45° C. and that the sample be shaken until the butter is of a thin creamy consistency. In addition to carrying out the ordinary bacteriological analysis, one cc. of the melted butter is added to 9 cc. of sterile water held at 40° C. to 45° C. and the contents thoroughly mixed. One cc. of this dilution is then added to two tubes of sterile litmus milk. One tube is incubated at 21° C. and the other test-tube at 37° C.

If there is coagulation of the litmus milk with production of acid and gas, the positive tube is streaked on an Eosin-Methylene-blue plate to confirm the presence of the Escherichia-Aerobacter group. In our work the litmus milk has proved a better enrichment medium than nutrient lactose broth for the detection of the Escherichia-Aerobacter group in butter.

REFERENCES

(1) HAMMER, B. W., AND YALE, M. W. Development of the Escherichia-Aerobacter group of bacteria in butter. JOURNAL OF DAIRY SCIENCE 15: No. 3, p. 199. 1932.

(2) KESSLER, M. A., AND SWENARTON, J. C. Gentian violet lactose peptone bile for the detection of *B. coli* in milk. Jour. Bact. 14: No. 1, p. 47. 1927.

(3) KLIMMER, M., HAUPT, H., AND BORCHERS, F. Über das Vorkommen und die Bestimmung der Coli und Aerogenesbakterien in der Milch. Milchwirtsch, Forschgn. 9: p. 236. 1930.

(4) MUER, T. C., AND HARRIS, R. L. The value of brilliant green in eliminating errors due to anaerobes in the presumptive test for *B. coli*. Amer. Jour. Pub Health 10: p. 874. 1920.

STUDIES ON THE IODINE CONTENT OF MILK

I. EFFECT OF DESICCATION AND STORAGE

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The importance of small amounts of iodine in nutrition seems to have been fully demonstrated by numerous investigators. Such data as are available indicate that milk normally contains small and highly variable amounts of iodine; the amounts reported ranging from traces to 276 parts per billion (1, 2, 3, 4 and 5). That such variations may be due primarily to the amount of iodine ingested in the ration is clearly shown by the work of Scharrer and Schwaibold (6), Krauss and Monroe (7) and by Watson (8). While the analytical difficulties involved in the quantitative estimation of the small amounts of iodine usually found in milk may preclude full acceptance of absolute values recorded, the general conclusions can hardly be contradicted.

The nature of the element and lack of knowledge regarding its exact combination in milk presents certain speculative questions as to whether iodine, either that existing as a normal constituent in milk or that which might be subsequently added, is retained after the milk is subjected to certain common procedures. Magee and Glennie (3) have reported that 20 per cent or more of the iodine content of milk is lost by heating.

Data here presented show the iodine content of milk before and after desiccation by the atmospheric double roller drying process; also the comparative iodine content of fluid and desiccated milks to which elemental iodine in suitable form and in definite amounts had been added previous to drying.

EXPERIMENTAL

The plan of experiment involved iodine determinations of parallel samples of fluid and dried milk to which elemental iodine had been added in known amounts before desiccation. It is rational to suppose that iodine added as the element would be more readily lost in the drying process than if added as iodide. This procedure was therefore used in order to make the conditions of the experiment as drastic as possible. The iodine undoubtedly unites at once to a large extent with the protein of the milk. A

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single batch of several hundred pounds of milk was divided into a number of smaller portions which were held at 70° to 75° F. for various periods of time until different degrees of acidity were developed. Portions of certain of the samples were conserved for subsequent analysis by the addition of sodium hydroxide and slow evaporation at 60° to 80° C. during a period of 24 to 36 hours, other portions of the same samples being dried by the roller process. In order to analyze milk for iodine, it is necessary that the sample be dried in some manner before combustion. Drying at 60–80°, after addition of alkali, was adopted for the control experiments as being least likely to involve losses of iodine. That iodine is not lost by heating at 80° in the presence of sodium hydroxide has been shown by Baumann (9). This parallel series of samples contained only the iodine inherent in the milk.

To other portions of milk of the same acidities, elemental iodine was added at the calculated rate of 2000 parts per billion of milk solids. This was accomplished by adding the proper amount of an assayed iodine solution directly to the milk during violent agitation and continuing the agitation for a few minutes. The strength of the iodine solution was determined by titration against N/100 sodium thiosulphate which had previously been standardized against iodine. Samples of the iodized fluid milk were conserved for later analysis in the same manner as were the uniodized samples. The remainder of the iodized samples were dried by the roller process. All desiccated samples were packed in hermetically sealed containers immediately after drying.

Although it was intended to add an exact 2000 parts of iodine per billion parts of milk solids, a careful appraisal of all possibilities of error was made for calculating the possible iodine content if all the errors were:

(a) Acting to increase concentration of iodine in the milk.

(b) Acting to decrease the concentration of iodine in the milk.

While it is improbable that all of the possible mechanical errors operative for a particular sample were of like action, the extremes of concentration which might exist lay between 1931 and 2174 parts per billion. The amount of iodine found in the uniodized fluid milk was taken into consideration for calculating the possible extremes of total iodine content of the samples to which iodine had been added. The calculations indicate that the total iodine content of such samples could not have been less than 2344 parts per billion nor more than 2625 parts per billion, dry basis.

A modification of the McClendon-Remington method of analysis (10, 5) was used with the exercise of such precautions as experience with the method seems to require. The Karns (11) method as modified by von Kolnitz and Remington (12) was used for a few check determinations with results substantially equivalent to those obtained by the McClendon-Remington method. The records in the accompanying table are the result of sev-

eral determinations made on each sample and include only those which showed reasonably close agreement. Low, and often entirely negative recoveries were obtained in many instances but in the majority of such cases one or more elements of mismanagement or inadvertency occurred in the manipulations which could account for the low result. It is to be noted that during the course of the work a possible serious source of error was indicated by the presence of chlorine gas in the atmosphere and by traces of chlorine in the distilled water obtained from an over-chlorinated municipal water supply. Although positive proof that such chlorine was responsible for many of the low recoveries is lacking, higher recoveries for subsequent estimations were substantially concurrent with the removal of the chlorine from the distilled water by activated granular carbon and with the elimination of chlorine from the atmosphere.

The data for comparable samples of milk dried by the commercial roller process, and the milk slowly evaporated at 60°–80° C. after the addition of alkali indicate that there is no siginficant loss of iodine due to the desiccating process under consideration. The percentage recovery from both series

TABLE 1

The recovery of iodine from liquid and dry milk

SAMPLE	METHOD OF DRYING	TITRABLE ACIDITY (cc. n/10 alkali per 100 cc. milk)	EXTREME LIMITS OF IODINE ADDED	EXTREME LIMITS OF TOTAL IODINE IN SAMPLE	IODINE RECOVERED	LIMITS OF RECOVERY
			parts per billion, dry basis			*per cent*
I	With alkali, 80°	14.8	None	———	392	———
IA	Roller Process	———	None	———	350	89.3
IB	Roller Process	14.8	1936–2174	2347–2635	2052	78–87
2A	Roller Process	16.3	1934–2174	2344–2635	2166	82–92
3	With alkali, 80°	18.5	1931–2174	2340–2635	1952	74–85
3A	Roller Process	———	1931–2174	2340–2635	2074	79–89
4	With alkali, 80°	20.8	1927–2174	2336–2635	2126	81–91
4A	Roller Process	———	1927–2174	2336–2635	2026	77–87

of samples was substantially the same. This clearly shows that recoveries less than the calculated amount must be due to some factor or combination of factors which affect both methods of drying, such as losses of free iodine during the process of addition to the milk, or flaws in the method of analysis. Variations in titratable acidity of the fluid milk ranging from 0.148 to 0.208 per cent, calculated as N/10 acid, did not appear to affect the recovery of iodine from either of the sets of samples. Samples of the desiccated product analyzed at intervals during a storage period of seven months did not show significant differences in iodine content in comparison with results obtained soon after preparation.

TABLE 2
Effect of storage on iodine content of dry milk

TIME OF STORAGE	IODINE CONTENT
0	2052
1 month	1918–1952
4 months	2072
7 months	1900

CONCLUSIONS

1. The iodine content of milk dried by the atmospheric double roller process is not less than that of milk slowly evaporated to dryness at 60–80° C. after the addition of sodium hydroxide. An average recovery of about 83 per cent was obtained from both milks to which elemental iodine had been added prior to drying.

2. Change of acidity within a range suitable for drying milk by the roller process does not affect the recovery of added iodine.

3. No apparent loss of iodine from dry milk results from storage for as long as seven months.

REFERENCES

(1) VON FELLENBERG, T. Untersuchungen über das Vorkommen von Jod in der Natur. VI. Mitteilung: Ueber den Zusammenhang zwischen der Häufigkeit des Auftretens von Kropf. Biochem. Ztschr. 152, 141. 1924.

(2) MONROE, C. F. The possibility of producing iodized milk. Bi-monthly Bull. Ohio Agric. Exp. Sta. 13, No. 4: 155. 1928.

(3) MAGEE, H. E., and GLENNIE, A. E. Studies on the effect of heat on milk. IV. The iodine content. Biochem. Jour. 22: 11. 1928.

(4) ORR, J. B. and LEITSCH, I. Iodine in nutrition. Brit. Med. Res. Council, Sp. Report, No. 123. 1929.

(5) MCCLENDON, J. F., REMINGTON, R. E., VON KOLNITZ, H. and RUFF, R. The determination of traces of iodine. III. Iodine in milk, butter oil and urine. Jour. Am. Chem. Soc. 52: 541. 1930.

(6) SCHARRER, K. and SCHWAIBOLD, J. The iodine content of milk. Biochem. Ztschr. 207, 333. 1929.

(7) KRAUSS, W. E. and MONROE, C. F. A comparison of the influence of iodized milk and potassium iodide administered directly, on the size and iodine content of the thyroid gland of rats. Jour. Biol. Chem. 89: 581. 1930.

(8) WATSON, C. J. A study of the reactions involved in the determination of minute traces of iodine, and the application of some feeding experiments with fishmeal. Thesis Cornell Univ. Library. 1928.

(9) BAUMANN, E. J. and METZGER, N. The determination of iodine in blood, foods and urine. V. Jour. Biol. Chem. 98: 413. 1932.

(10) REMINGTON, R. E., MCCLENDON, J. F. and VON KOLNITZ, H. The determination of traces of iodine. V. Further refinements in technique. Jour. Am. Chem. Soc. 53: 1245. 1931.

(11) KARNS, G. M. Ashing apparatus for samples containing traces of iodine. Ind. and Eng. Chem., Anal. Ed., 4: 299. 1932.

(12) VON KOLNITZ, H. and REMINGTON, R. E. A simplified Karns technique for the micro-estimation of iodine. Ind. and Eng. Chem., Anal. Ed., 5: 38. 1933.

STUDIES ON THE IODINE CONTENT OF MILK

II. VARIATIONS IN THE MIXED MILK OF HERDS

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INTRODUCTION

When compounds of iodine are ingested by lactating animals, a part of the iodine appears in the milk. For pharmacological doses, this has been known for many years (1). That it is likewise true for those smaller amounts which occur in normal nutrition, and that the iodine of the milk is increased by an increase in that ingested, has been shown by Scharrer (2) and by Krauss (3). It seems evident that iodine is a normal constituent of milk, being correlated in amount to the available iodine content of the ration of the cows.

It has been shown by Hayne (4) that diseases due to iodine deficiency are not endemic in South Carolina, and by Remington (5) that crops which are produced there contain more iodine than those from more goitrous areas. In estimating the iodine available to the population in this area, it is necessary to include some studies on milk.

Many of the values found in the literature for the less common elements in foods (and for vitamins as well), are based on a single sample, taken without regard to locality, season, or conditions of growth. The work of the senior author (6) and his associates has brought out that wide variations exist, not alone with regard to iodine, but also with regard to iron, copper, and manganese, and not only in agricultural crops, but sea food as well (7); hence the acceptance of values based on one, or but few samples, may give rise to erroneous conclusions.

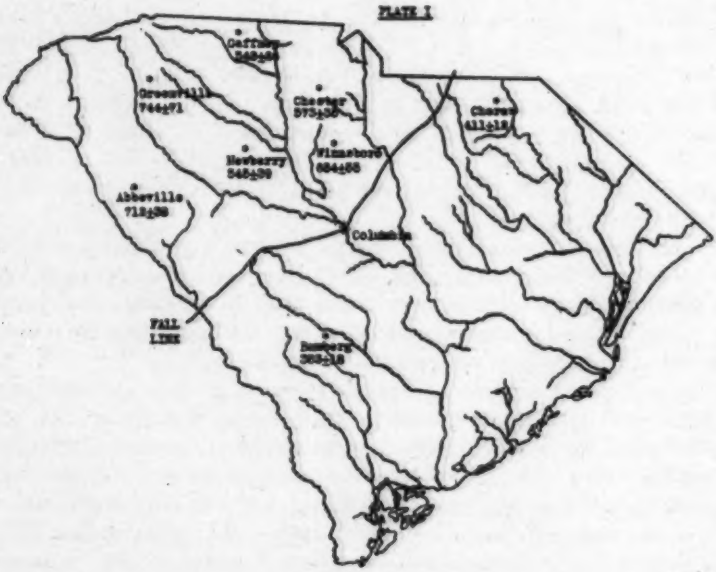
In the present investigation, the aim is to determine what variations may exist in the iodine content of the mixed milk of herds from a given area, and to correlate such variations as may be found with locality, season of the year, or any other factors which may be observed.

PLAN OF THE INVESTIGATION

Through the cooperation of the Dry Milk Company, an experimental milk drying plant was installed in the territory. This plant was of the roller process type, and operated by an experienced technician furnished

by the Company. The method of handling and drying was made to conform to that in usual commercial practise. A portion of the fat of the milk was removed prior to drying. This could not materially affect the iodine content, it having been shown by Scharrer (2) that not over 4 per cent of the total iodine of milk is normally contained in the fat. That the iodine content of milk is not materially reduced by this method of drying has been shown in the preceding paper (8).

Eight collecting points were selected, two lying near the foot of the Blue Ridge of the Appalachian Mountains, four in the rolling country between the Blue Ridge and the Coastal Plain, and two in the Coastal Plain (See map, Plate I). Milk was collected in batches of approximately 1,000



pounds, three times a week, the collecting points being handled in rotation. Each point, therefore, was visited at intervals of three weeks, one collecting date on each circuit being left vacant for shop and office work in the plant. In selecting herds for the experiment, it was specified that only cattle receiving none but home-grown feeds were desired. The infrequent collections, from any given point, and the restriction as to the use of feeds imported from other regions made it necessary to draw the samples from small herds. On this account, the number of dairymen contributing to each sample of milk ranged from seven to nineteen, as follows:

	Number of herds contributing
Abbeville	19
Bamberg	7
Cheraw	14
Chester	7
Gaffney	15
Greenville	7
Newberry	7
Winnsboro	13

It was originally planned that the collection and drying of the milk samples should extend over a period of one year, but due to reasons not pertinent to this discussion, it was terminated at the end of ten months. The first collection was made on October 26, 1931, the last on September 2, 1932. Four times during the course of the experiment, a dairy specialist from the laboratory made the complete circuit, visiting every farmer, and obtained data as to methods of feeding, and samples of all feed and forage used. During the course of the project, fifteen samples were collected from each of five points, fourteen from each of the other three.

Samples of all batches of milk powder produced were sent to the laboratory as promptly as possible. Without further drying or manipulation, these were analyzed for iodine. The analytical procedure at the beginning of the study was that devised by McClendon as modified by Remington and associates (9). This method, while the best available for large samples at that time, is extremely laborious, and gives frequent failures which cannot with certainty be attributed to any known cause. In April there became available to us a manuscript by Karns (10), describing a method of ashing in a closed bulb which seemed to offer distinct advantages. There was accordingly devised a simplified type (11) of the Karns apparatus, and after experimental work on the recovery of known amounts of iodine, the method was adopted as routine on subsequent samples. Following this change, all analyses previously made by the McClendon method were repeated, using the newer technique, with the result that the average recovery of iodine was about ten per cent higher than had previously been obtained.

Since the method of final estimation is an isolation and colorimetric one, and since possibility of contamination by reagents has been rigorously ruled out, the only errors possible are those due to loss of iodine in the process of burning and subsequent manipulation of the samples. The *highest value* that can be reproduced within a maximum limit of variation of ten per cent on a repeat analysis, is, therefore, taken as the most nearly correct.

GENERAL RESULTS

The analytical work embraced 117 samples from the eight collecting points in South Carolina, 9 samples during the same period taken from the regular run of a commercial dry milk plant at Bainbridge, N. Y., and 6

TABLE 1

Iodine content of milk samples, collected in South Carolina, arranged as to points and dates of collection

(Parts per Billion, Dry Basis)

	ABBEVILLE	BAMBERG	CHERAW	CHESTER	GAFFNEY	GREENVILLE	NEWBERRY	WINNSBORO	MEAN	P.E.M.
Oct. 26–Nov. 13	828	336	370	892	804	.....	338	724	613	± 64
Nov. 16–Dec. 4	616	384	456	436	680	628	447	885	566	± 59
Dec. 7–Dec. 26	828	456	476	672	.....	600	472	844	621	± 42
Dec. 28–Jan. 15	852	412	349	546	668	424	576	837	583	± 45
Jan. 18–Feb. 5	424	412	444	584	376	466	468	1041	527	± 52
Feb. 8–Feb. 26	668	428	363	536	652	457	592	917	577	± 38
Feb. 29–Mar. 18	624	440	464	758	556	585	1080	1432	742	± 75
Mar. 21–Apr. 8	588	264	432	1080	600	492	1056	668	648	± 69
Apr. 11–Apr. 29	552	440	432	400	472	492	500	496	473	± 11
May 2–May 20	474	224	300	333	500	496	446	497	409	± 26
May 23–June 10	548	143	376	428	420	840	428	324	438	± 47
June 13–July 1	1120	244	324	684	279	1084	311	356	550	± 87
July 2–July 22	636	440	520	420	536	972	484	492	563	± 42
July 25–Aug. 12	760	.....	492	432	452	1008	420	388	565	± 59
Aug. 15–Sept. 2	1170	320	360	400	680	1872	560	360	715	±130
Mean	712	353	411	573	548	744	545	684	572	
P.E.M.	±38	±18	±12	±35	±26	±71	±39	±55		± 16

TABLE 2

Iodine content of milk produced at Bainbridge, N. Y., and Columbus, Wisconsin

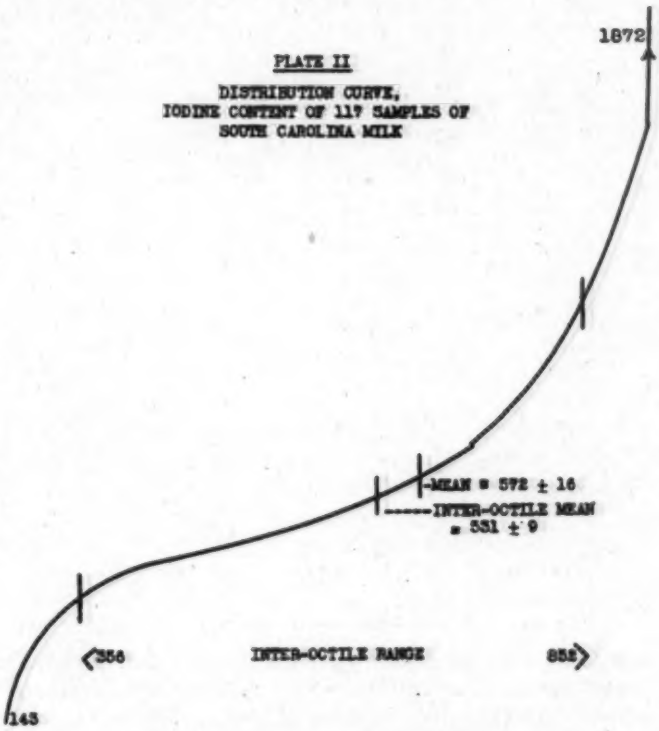
(Parts per Billion, Dry Basis)

BAINBRIDGE		COLUMBUS	
date	*iodine*	*date*	*iodine*
1–16–32	389	1– 7–30	388
4– 8–32	235		
4–15–32	245	4– 8–30	316
4–22–32	335	4–17–30	395
6–10–32	131	6–16–30	171
9–10–31	157	9– 9–30	338
9–11–31	147		
12–21–31	392	12– 7–30	326
12–17–31	350		

samples similarly obtained at Columbus, Wisconsin. The South Carolina samples, as shown in detail in table 1, ranged in iodine content from 143 to 1,872 parts per billion, with a mean value of 572 ± 16.

The distribution curve is shown in Plate II. If the upper and lower octiles be discarded, the range is from 356 to 852, mean 531 ± 9.

The range of values from Bainbridge, N. Y. (Table 2), was 392 to 131, mean 265, and from Wisconsin 395 to 171, mean 322.



RELATION TO PLACE OF PRODUCTION

The mean of all samples from each collecting point has been determined, and treated statistically according to accepted methods (see Sherman, Chemistry of Foods and Nutrition, Appendix D, for a simple explanation) in order to determine the probable errors of the means, and significance ratios for their differences. In table 3, the upper figure in each block is the difference between the average values for the two points concerned, the lower figure the significance ratio obtained as above, from which it becomes apparent that while Cheraw and Bamberg are scarcely significantly

different from each other, they are both significantly lower than all other points.

TABLE 3

Significant data for average iodine content of milk from eight collecting points

The first figure in each square is the difference between the average values for the two points shown; the second, the significance ratio obtained by dividing this difference by the square root of the sum of the squares of the probable errors of the two averages.

	BAMBERG	CHERAW	CHESTER	GAFFNEY	GREENVILLE	NEWBERRY	WINNSBORO
ABBEVILLE 712 ± 38	359 8.5	301 7.5	139 2.7	164 3.6	32 0.4	167 3.1	28 0.4
BAMBERG 353 ± 18		58 2.6	220 5.6	195 6.1	391 5.3	192 4.5	331 5.7
CHERAW 411 ± 12			162 4.4	137 4.7	333 4.6	134 3.3	273 4.9
CHESTER 573 ± 35				25 0.6	171 2.2	28 0.5	111 1.7
GAFFNEY 548 ± 26					196 2.6	3 0.1	136 2.2
GREENVILLE 744 ± 71						199 2.5	60 0.6
NEWBERRY 545 ±39							139 2.1
WINNSBORO 684 ± 55							

The two points showing highest values, Abbeville and Greenville, are scarcely significantly different from other points except Bamberg and Cheraw, with the possible exception of the difference between Abbeville and Gaffney, which shows a ratio of 3.6.

This higher iodine content of milk from points in the Piedmont as compared with the Coastal Plain, is in agreement with previously published data on potatoes (5), and with many unpublished results on leafy vegetables. It apparently contradicts the salt spray theory of iodine distribution, and suggests a source of iodine enrichment inherent in the origin of soils in this region.

RELATION TO SEASON

Inspection of average values for individual collection periods (Table 1) reveals a rather definite fall in iodine content during April and May, fol-

lowed by recovery in mid-summer. This period of lower values covered three circuits, from April 11 to June 10. If these three circuits are averaged together, the preceding eight circuits averaged in two groups of four each, and the following four circuits treated as one group, the following mean values are obtained:

Oct. 26–Jan. 15	30 samples	595 ± 23
Jan. 18–Apr. 8	32 samples	623 ± 32
Apr. 11–June 10	24 samples	440 ± 33
June 13–Sept. 2	31 samples	599 ± 43

There appears to be a significant difference between the second and third groups (ratio 3.9) and between the third and fourth (ratio 2.9). If, however, we discard the highest and lowest octiles and then treat the data in the same way, the following results are obtained:

Oct. 26–Jan. 15	25 samples	601 ± 22
Jan. 18–Apr. 8	25 samples	523 ± 14
Apr. 11–June 10	19 samples	486 ± 15
June 13–Sept. 2	20 samples	494 ± 18

The only significant difference here is that between the first group and each of the others. Probably about all we can say is that there is a slight definite decrease in iodine content in the spring, followed by a return to higher values in late summer or fall.

Of the 9 samples from Bainbridge, the highest values were obtained in December and January, the lowest in mid-summer (June to September), and the difference is much greater than in the South Carolina series. If a similar drop occurred at Columbus, it is evidenced by only one sample (June) of the six analyzed. Although but few analyses are given for these northern points, they represent large batches from many producers, and hence should be compared with averages rather than individual values in the South Carolina series.

The results indicate that the iodine content of milk in South Carolina is higher throughout the year and less subject to seasonal fluctuation, than that of Central New York or Wisconsin.

RELATION TO DIET

In an attempt to find a relationship between the iodine content of the milk and that of the ration, each South Carolina producer was visited four times during the collection period, *viz.*, in January, March, May, and August. At each visit, samples were taken of all materials fed, and information obtained as to kind of salt used, and source of water supply. From the samples obtained, and making use of information gained at time of collection, a composite sample representing the complete ration of each herd was constructed. These were later combined, in proportion to the amount

of milk contributed by each herd to a single batch of milk, so as to make a feed sample which corresponded to the milk sample for the given place and date. There were thus obtained 32 composite feed samples, 4 from each point, which could be compared as to iodine content with the milk samples.

Of the 89 herds, 3 received rock salt, 12 Morton's Stock Salt (non-iodized), and the balance common salt. The water supply was almost universally from surface waters, 75 per cent being from springs, streams and ponds, and 25 per cent from shallow wells. In January six herds in five localities received commercial mixed feeds in small amount. In two of these the formula showed 1 per cent of iodized salt. The number using commercial feeds fell to five in May, and three in March and August.

The principal concentrate used generally was cottonseed or cottonseed products, which for all herds amounted to 24 per cent of the dry weight of the ration in January, 27 per cent in March, 18 per cent in May, and 15 per cent in August. Next in importance among the concentrates was corn, accounting for 9, 8, 4, and 3 per cent of the ration respectively in the different periods. Legume hays, mainly pea and soy bean, were fed to the extent of 29, 23, 4, and 3 per cent, and other roughages, mostly corn and sorghum hay and silage 34, 34, 15, and 10 per cent. Although cows range out of doors the year round, no grazing was reported in January, 5 per cent in March, 58 per cent in May, and 63 per cent in August.

During the period when fresh pasturage contributed materially to the ration, all but a very few herds were on permanent pasture, the basis of which was Bermuda grass, usually with one or more legumes. In May 65 per cent of the pastures contained, in addition to grasses, some form of clover, such as burr, hop, or white clover, lespadeza or alfalfa, many containing several varieties together. In August, on the other hand, 82 per cent of pastures contained legumes, in this case mostly lespedeza.

The average iodine content of the 32 rations was found to be 475 parts per billion, but the range of values is almost as wide as for the milk samples. While those feed samples showing very high values produced milk of similar iodine content, in general no correlation could be found between the feed and the milk. In fact, it is apparent that the experiment is not sufficiently controlled, since we have been forced to rely on the statements of herdsmen as to the qualitative and quantitative make-up of the ration; the factor of accidental contamination of ration or milk (as, for instance, by the use of tincture of iodine as an antiseptic on the person, or on cows themselves) has not been guarded against; and no account could be taken of physiologic factors which might influence the metabolism of iodine by the animals.

A number of random samples of feeds, taken from different parts of the area studied, however, reveal that the legume hays are richest in iodine, and that grain in general is very poor (Table 4).

TABLE 4

Iodine content of some feeds produced in South Carolina

FEED	NO. SAMPLES	IODINE PARTS PER BILLION DRY BASIS
	Concentrates	
Cottonseed Meal	4	151
Corn	3	10
Oats	1	20
Wheat	2	17
	Legume Hays	
Peavine	11	522
Soy Bean	14	526
Lespedeza	2	446
Alfalfa	7	429
Vetch	8	425
Sweet Clover	2	320
	Other Hays and Roughage	
Cottonseed Hulls	5	107
Oat Hay	2	116
Mixed Meadow Hay	3	263
Cane Stover	1	340
Corn Stover	1	161

Although the number of samples reported in this table is insufficient for firm conclusions, it appears probable that legume pasture and hay is the largest contributor of iodine to the ration of dairy cows in this locality.

SUMMARY

Samples of mixed milk of several herds at each of eight different points in South Carolina, were collected at intervals of three weeks, over a period of ten months (November, 1931, through August, 1932) and dried by the roller process. The cattle received only locally grown foods and feeds, and none were given iodized salt.

Iodine estimations on the dried milk were made by the method of McClendon as modified by Remington, and also by von Kolnitz' adaptation of the Karns technique, the latter method proving shorter and more workable, besides giving about 10 per cent higher recoveries.

The average iodine content of the 117 samples was 572 ± 16 parts per billion, dry basis. Averages for two points in the coastal plain (Bamberg 353 ± 18, Cheraw 411 ± 12) were significantly lower than for six points in the Piedmont (Abbeville 712 ± 38, Chester 573 ± 35, Gaffney 548 ± 26, Greenville 744 ± 71, Newberry 545 ± 39, and Winnsboro 684 ± 55). Values obtained in April and May were slightly but significantly lower than for the remainder of the period.

During the same period nine samples taken at a commercial milk drying plant in New York, and six samples at one in Wisconsin, averaged respectively 265 ± 24, and 322 ± 22; and seasonal variations were much greater than for the South Carolina samples.

REFERENCES

(1) KOLDA, J. Du passage des substances medicamenteuse dans Le Lait. Le Lait 6: 12 and 269. 1926.

(2) SCHARRER, K., and SCHWAIBOLD, J. The iodine content of milk. Biochem. Zeit. 207: 333. 1929.

(3) KRAUSS, W. E. A comparison of the influence of iodised milk and potassium iodide administered directly, on the size and iodine content of the thyroid gland of rats. J. Biol. Chem. 89: 581. 1930.

(4) HAYNE, J. A. Endemic goiter and its relation to the iodine content of food. Amer. J. Public Health 19: 1111. 1929.

(5) REMINGTON, R. E., CULP, F. B., and VON KOLNITZ, H. The potato as an index of iodine distribution. J. Amer. Chem. Soc. 51: 2942. 1929.

(6) REMINGTON, R. E., and SHIVER, H. E. Iron, copper and manganese content of some common vegetable foods. J. Assoc. Official Agric. Chemists 13: 129. 1930.

(7) COULSON, E. J., LEVINE, H., and REMINGTON, R. E. Oysters and Anemia. Amer. J. Public Health 22: 1141. 1932. (Also unpublished data on the iodine content of oysters.)

(8) HANFORD, Z. M., SUPPLEE, G. C., and REMINGTON, R. E. Studies on the iodine content of milk. I. Effect of desiccation and storage. JOURNAL OF DAIRY SCIENCE 17: 15–18. 1934.

(9) REMINGTON, R. E., MCCLENDON, J. F., and VON KOLNITZ, H. The determination of traces of iodine. V. Further refinements in technique. J. Amer. Chem. Soc. 53: 1245. 1931.

(10) KARNS, G. M. Ashing apparatus for samples containing traces of iodine. Ind. and Eng. Chem., Anal. Ed., 4: 299. 1932.

(11) VON KOLNITZ, H., and REMINGTON, R. E. A simplified Karns technique for the micro-estimation of iodine. Ind. and Eng. Chem., Anal. Ed., 5: 38. 1933.

SUBSTANCES ADSORBED ON THE FAT GLOBULES IN CREAM AND THEIR RELATION TO CHURNING. III. ANALYSIS OF THE ADSORBED PROTEIN*

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Protein constitutes the major part of the material adsorbed on the surface of the fat globules in cow's milk (1). The protein has been identified by various workers as a glycoprotein (2), casein (3), albumin (4), a globulin-like protein (5), and a new milk protein (6).

Some of the observed difference in composition may have resulted from the method used in the isolation of the material. A method whereby the stabilized fat globules in milk are freed from milk plasma by allowing the globules to rise through a single comparatively short column of water does not prevent the occlusion of plasma protein by the clusters of fat. A much more drastic procedure was used in the preparation of the material analyzed in the present study. The detailed method has been described in two preceding papers (1, 7). The general procedure was as follows.

Fresh heavy cream was repeatedly diluted with distilled water and passed through a centrifugal separator until the "skim milk" or wash water was free from milk plasma solids. The so-called washed cream was then churned to remove the major part of the fat. The butter was melted and the material which had remained adsorbed on the surface of the fat during the washing operation was recovered in the buttermilk and aqueous layer from the melted butter. These were concentrated and the protein fraction precipitated from the concentrate at pH 3.9–4.0. After filtration the protein was exhaustively extracted with alcohol, chloroform, and ether.

The results of analysis for total nitrogen, sulfur, and phosphorus have been reported (7). The following table also shows the nitrogen distribution analysis. The standard Van Slyke procedure described by Morrow (12) was followed. Corrections for the solubility of the basic phosphotungstates were not made inasmuch as they were filtered at a temperature of 40° F. For comparative purposes, the analyses of other milk proteins are included in the table.

It will be noted from the table that the "membrane" protein from washed cream differs from other milk proteins in all respects. The most striking difference lies in the nitrogen fractions. In this regard, it agrees

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Composition of Milk Proteins

NITROGEN DISTRIBUTION	MEMBRANE PROTEIN			CASEIN[a]	LACT-ALBUMIN[a]	GLOBULIN[a]	ALCOHOL-SOLUBLE PROTEIN		HAPTEIN (6)
	I	II	Ave.				(9)	(10)	
Ammonia-N	6.35	6.73	6.54	10.36	8.25	7.64	1.57	10.9	9.98
Insoluble humin-N	3.22	3.23	3.23	1.90	2.32	2.16	.24	.35	3.48
Soluble humin-N	0.43	0.44	0.44	.....	.....	.....	.....	.....	.....
P.T.A. humin-N	.....	0.20	0.20	.....	.....	.....	.....	.....	.....
Total humin-N	3.85	3.87	3.86	.....	.....	.....	.....	.....	.....
Arginine-N	15.54	16.09	15.82	7.90	7.38	10.81	5.98[b]	8.30	12.79
Cystine-N	0.78	0.65	0.72	0.81	1.74	1.90	.....	.....	5.34
Histidine-N	2.26	1.11	1.69	5.85	4.51	3.99	3.93[b]	2.00	5.44
Lysine-N	11.01	11.97	11.49	9.26	12.39	8.66	4.87[b]	.....	6.11
Basic NH_2-N	16.43	17.02	16.73	.....	.....	.....	.....	.....	.....
Basic non-NH_2-N	13.16	12.80	12.98	.....	.....	.....	.....	.....	.....
Total basic-N	29.59	29.82	29.71	23.82	26.02	25.35	.....	.....	29.68
Non basic NH_2-N	56.41	56.58	56.50	56.74	60.92	62.82	.....	.....	55.13
Non basic non-NH_2-N	3.55	3.46	3.51	7.50	2.33	1.14	.....	.....	1.61
Total non basic-N	59.96	60.04	60.01	64.22	63.25	63.96	.....	.....	56.74
Total recovery	99.75	100.46	100.12	100.32	99.83	99.10	.....	.....	99.88
Percentage total N	11.84	12.64	12.22	15.67	15.43(11)	15.44(11)	15.71	15.49	12.05
Percentage total S	0.90	1.02	0.96	0.72	1.92	0.86	.95	.....	2.58
Percentage total P	0.24	0.51	0.33	0.85	trace	0.24	.08	.108	.....

[a] Average of summarized data (8).
[b] Calculated from percentage of amino acid reported present.

with haptein, the "membrane" protein isolated by Hattori (6). Haptein and the washed cream protein are low in the percentage of total nitrogen and high in the percentage of humin or melanin nitrogen and total basic nitrogen as compared with casein, lactalbumin and globulin. The former two proteins, however, differ greatly in the nitrogen distribution within the basic fraction. Haptein is characterized by a high percentage of arginine and cystine whereas the washed cream protein is characterized by a high percentage of arginine but low percentages of cystine and histidine. The total sulfur content of haptein is also high compared with other milk proteins.

It appears further from the physical properties of these two proteins (1, 6) that they are very nearly alike but differ somewhat in composition due to impurities which have resulted from two widely different methods of preparation. Moreover, the analyses show that the "membrane" protein of milk fat is not identical with casein, lactalbumin or globulin, or the alcohol soluble protein of milk.

The serological properties of the "membrane" protein are being investigated by Dr. Julian H. Lewis, Department of Pathology, University of Chicago. A preliminary report of this study was given at the Cincinnati meeting of the American Society of Experimental Pathology, April 10–12, 1933, by L. S. Palmer and Julian H. Lewis, under the title "The Serological Properties of a New Protein from Milk." The experiments reported indicate that the protein possesses specific biological properties.

REFERENCES

(1) PALMER, L. S. and WIESE, H. F. Substances adsorbed on the fat globules in cream and their relation to churning. II. The isolation and identification of absorbed substances. JOURNAL OF DAIRY SCIENCE 16: 41. 1933.

(2) STORCH, V. Consistensfejt hos Smörret. Den kgl. Veterinärog Landhohsjskoles Laboratium for landøkonomiske Forsøg. 36 Beretning. Ueber den Bau der Milchkügelchen. Abschnitt III Jahresber. Fortschritte Tierchem. 27: 273. 1897.

On structure of "fat globules" in cow's milk. Analyst 22: 197.

(3) TITUS, R. W., SOMMER, H. H. and HART, E. B. The Nature of the protein surrounding the fat globules in milk. Jour. Biol. Chem. 76: 237. 1928.

(4) BÉCHAMP, A. Sur les altérations spontanées du Lait et sur celles que la cuisson lui fait subir. Bul. Soc. Chim. 3rd Ser. 15: 426. 1896.

(5) PALMER, L. S. and SAMUELSSON, E. The nature of the substances adsorbed on the surface of the fat globules in cow's milk. Proc. Soc. Exp. Biol. Med. 21: 537. 1924.

(6) HATTORI, K. Über die Hüllensubstanz der Milchfettkügelchen. Jour. Pharm. Soc. Japan No. 516, 123. 1925.

Beiträge zur Untersuchung über die Hüllensubstanz der Michfettkügelchen. Jour. Pharm. Soc. Japan 49: 332. 1929.

HATTORI, K. and OGIMURA, Y. Beiträge zur Untersuchung über die Hüllensubstanz der Milchfettkügelchen. II. Jour. Pharm. Soc. Japan 49: 1147. 1929.

(7) WIESE, H. F. and PALMER, L. S. Substances adsorbed on the fat globulins in cream and their relation to churning. I. The churnability of artificial emulsions prepared with known emulsifying agents of cream. JOURNAL OF DAIRY SCIENCE 15: 371. 1932.

(8) LAMOUR, R. K. Correlations between the total nitrogen of the bases and arginine nitrogen and between total nitrogen of the bases and lysine nitrogen, of various proteins. Trans. Roy. Soc. Can. 22: 349 (section 5). 1928.

(9) OSBORNE, T. B. and WAKEMAN, A. J. Some new constituents of milk. A new protein, soluble in alcohol. Jour. Biol. Chem. 33: 243. 1918.

(10) LINDERSTRØM-LANG, K. Studies on casein. III. The fractionation of casein. Comp. rend. trav. lab. Carlsberg 17 No. 9. 1929.

(11) OSBORNE, T. B. and WAKEMAN, A. J. The proteins of cow's milk. Jour. Biol. Chem. 33: 7. 1918.

(12) MORROW, C. A. Biochemical laboratory methods for students of the biological sciences. John Wiley and Sons, Inc., New York. 1927.

A STUDY OF THE RELATIONSHIPS BETWEEN HYDROGEN ION CONCENTRATION, TITRATRABLE ACIDITY, AND QUALITY IN CHEDDAR CHEESE

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The manufacture of sour or acid cheese causes serious losses each year to the American cheese industry. The excessive acidity may affect any or all of the properties of the cheese, such as flavor, body, texture, color and finish. The defect may be almost negligible or it may be highly injurious to the market value of the product.

Excessive acidity may be avoided by skillful operators through careful observation of the milk and curd from day to day. Tests which indicate, but do not measure acidity accurately, are the so-called "rennet test" and "hot-iron test." These methods of detecting acidity attain their greatest value when used every day by the same operator on the same milk supply. The alkali titration test is commonly used to measure acidity during the cheese-making process. Optimum degrees of titratable acidity in the starter, milk or whey have been indicated for nearly every operation. But defective cheese is sometimes made even though these schedules of optimum titratable acidities have been carefully observed.

It is well known that the titratable acidity of fresh, normal milk may vary through wide limits (1) due to the influence of a number of factors (2, 3, 4). The differences in titratable acidity which may be caused by the variations in the composition of milk should be particularly significant in the manufacture of cheese. Measurements of titratable acidity during the making process actually indicate, that the maximum acidities, which may be safely attained in the manufacture of cheese from milk with high solids content, may be disastrous if applied to milk which is low in solids-not-fat.

The hydrogen ion concentration furnishes an indication of changes in milk which can not be definitely measured by titratable acidity. For example, the maximum hydrogen ion concentration attained during the fermentation of milk by a given organism is quite constant; then, also, the changes in the casein of milk, which are caused by increasing acidity, culminate in coagulation at the iso-electric point of pH 4.7. It seems possible that measurements of hydrogen ion concentration might furnish a better means of detecting critical changes during the making of cheese than do measurements of titratable acidity.

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It is the purpose of this paper to report the results of a study of the titratable acidity and hydrogen ion concentration during the making of cheddar cheese and to indicate their relation to the quality.

Determination of hydrogen ion concentration

The hydrogen ion concentration measurements of the milk and whey were made with the Biilmann type of quinhydrone electrode. This electrode was adopted because only a small sample is required and the reading may be taken immediately. These qualifications are highly desirable in as much as the amount of material available for testing is limited at times during the process and because the acidity changes so rapidly that any delay in obtaining the measurement would introduce errors. A Leeds-Northrup, type K, potentiometer and a 0.1 normal calomel electrode were used in this study. All readings were made at 25° C.

The quinhydrone electrode described and used by Knudsen (5) and Watson (6) was adopted for use in this study for determining the pH of the cheese. Some difficulty was experienced with this electrode when it was first tried.[1] There was considerable "drifting" of the potential at times, especially with fresh curd, which made it impossible to take the reading with any degree of certainty. Special precautions were taken (7) to clean the electrode properly, but even this treatment did not eliminate the trouble entirely. Finally, it was found that the addition of a drop or two of distilled water to the mixture of about two grams of cheese and 50 milligrams of quinhydrone crystals eliminated the trouble with drifting potentials.

The hydrogen electrode was also used in attempts to measure the pH of the cheese. Samples of the cheese were ground and diluted with distilled water in the proportions of 1 part of cheese to 2, 5, 10 and 20 parts of water. The results were not entirely satisfactory because the degree of dilution had a marked effect on the pH value. The measurements obtained by diluting samples of several lots of cheese of different ages are shown in figure 1. The points on these curves which show no dilution were determined by the quinhydrone method. All other measurements were made with the hydrogen electrode.

Figure 1 indicates that the degree of dilution has a distinct influence on the pH value;—the greater the dilution, the higher is the pH. The change in pH caused by dilution is variable and apparently cannot be predicted from a single observation of an unknown sample of cheese. The measurements of the samples with the least dilution (1: 2) were made with diffi-

[1] The authors are grateful for the valuable assistance and advice of Dr. H. H. Sommer, of this University, Dr. Paul F. Sharp, of Cornell University, and Dr. P. D. Watson, of the Bureau of Dairy Industry, U. S. Department of Agriculture, in correcting this difficulty.

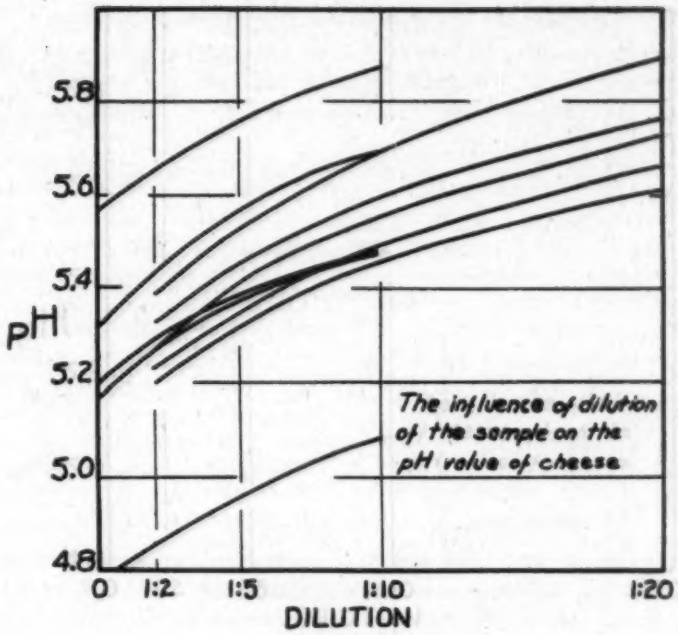

Figure 1

culty. The use of the hydrogen electrode seemed to be entirely unsuited for the purpose of this study.

Schulz (8) and Pasztor (9) have observed the same influence of dilution upon the pH of cheese. Schulz found no change in pH with the quinhydrone electrode as long as the dilution was kept below the ratio of 1:1. Pasztor reported an increase of approximately 0.50 pH between a dilution of 1:1 and 1:24.

Samples which were taken from various parts of the same cheese showed variations of not more than 0.02 pH when tested by the quinhydrone method. Some typical results of such trials are shown in table 1. An ordinary cheese trier was used to remove the sample and the half inch which included the rind was replaced in the cheese. The rest of the plug was ground in a mortar and a portion of the mixture used for the test. Schulz (8) observed a wide difference between the reaction of different parts of the interior of Tilsit cheese. These variations can probably be explained by the practice of salting this cheese from the outside and curing it under conditions which are not typical of cheddar cheese.

Determination of titratable acidity in milk, whey and cheese

The determination of titratable acidity in the milk and whey were made by the usual method of titrating 9 or 18 gram samples with 0.1 normal sodium hydroxide using phenolphthalein as indicator. The acidity was expressed as per cent lactic acid.

The A. O. A. C. method was used for determining the acidity of the cheese (10).

TABLE 1

Variations in the pH of samples taken from different portions of the same cheese

CHEESE NO.	AGE	LOCATION OF SAMPLE IN CHEESE			
		Center	Three points near the edge		
			1	2	3
	days	*pH*	*pH*	*pH*	*pH*
51	2	4.99	4.99	4.98	4.97
83	6	4.90	4.91	4.91	4.89
28	21	5.08	5.08	5.08	5.08

Manufacture of the experimental cheese

Paired lots of cheese were made from identical milk. One lot was made in a normal way while the second lot was manufactured in some manner to produce a sour cheese. A total of 54 lots were made in this manner. They were cured at a temperature of about 45° F.

The hydrogen ion concentration and titratable acidity measurements were made at the critical points of the curd-making process. These points included the times of receiving the milk, adding the rennet, cutting the curd, beginning and ending the heating process, removing the whey, and packing, milling and pressing the curd. The pH and titratable acidity measurements were made on the milk until it was coagulated. When it was necessary to do so, the soft coagulum was used for pH measurements until the curd was cut. All acidity measurements were made on the whey between the operations of cutting and milling the curd. Determinations of pH were made on the curd at milling and pressing.

Acidity and pH measurements were made on the cheese when it was removed from the press, once each day for 7 days after pressing, and then at the ages of 14, 21, 35, 49 and 70 days, and approximately 9, 12 and 24 months.

The cheese were scored after 2 months of curing. The score card allowed 30 points for flavor, 40 for body and texture, 10 for color, and 20 for make-up. The scores of three judges were averaged to obtain the recorded score for each cheese. The cheese were scored again in the same manner at approximately 10 months.

EXPERIMENTAL RESULTS

The 54 lots of cheese were divided into three groups according to their quality at two months of age. Group I consisted of 14 lots scoring 90 or above; group II contained 25 lots scoring from 87 to 90, while group III was composed of 15 lots with scores of less than 87. At two months of age the average scores of the cheese in groups I, II and III were 91.1, 88.7, and 84.8, respectively. After 10 months of curing the average scores of the cheese in these groups named in the same order were 90.2, 88,7, and 86.3. These figures indicate some deterioration in the average quality of the cheese in group I and improvement in quality of the cheese in group III.

It is believed from a careful study of the ripening of cheddar cheese that the scores at the age of two months represent more accurately than the 10 months' score the influence of acidity on the properties of the cheese. After 10 months of curing, the changes in the cheese which are caused by those organisms and enzymes that are responsible for protein decomposition, tend to disguise the effects of excess acid.

Acidity measurements during the curd-making process

The average pH values at the various steps in the curd-making process are shown with their respective probable errors in table 2. These average values were calculated directly from the pH measurements. Averaging the pH values gave practically the same result as would have been obtained if the acidity had been stated in terms of hydrogen ion concentration and these values then averaged and the average converted into pH. For example, the pH measurements of 13 lots of cheese at the intervals of cutting, 3 days after pressing and 9 months after making, were averaged separately and found to be 6.473, 5.045 and 5.323, respectively. The same acidity measurements were then converted to hydrogen ion concentrations and the averages calculated. These averages were then changed back to pH with the result that the mean was found to be 6.469 at cutting; 5.032 at 3 days of age; and 5.321 at 9 months of age.

Table 3 presents the average titratable acidity measurements at the various steps in the curdmaking process.

Tables 2 and 3 indicate slight but noticeable differences in acidity between the three groups at the time of adding the rennet to the milk. These differences in both titratable acidity and pH measurements become increasingly evident as the cheese making process continues. There is a definite rise in pH in the interval between the addition of the rennet and immediately after coagulation of the curd. This increase is not gradual during this interval but apparently occurs at the moment of coagulation, since tests made immediately before and after visible coagulation showed increases in some instances as great as 0.15 pH. It is interesting to speculate on the

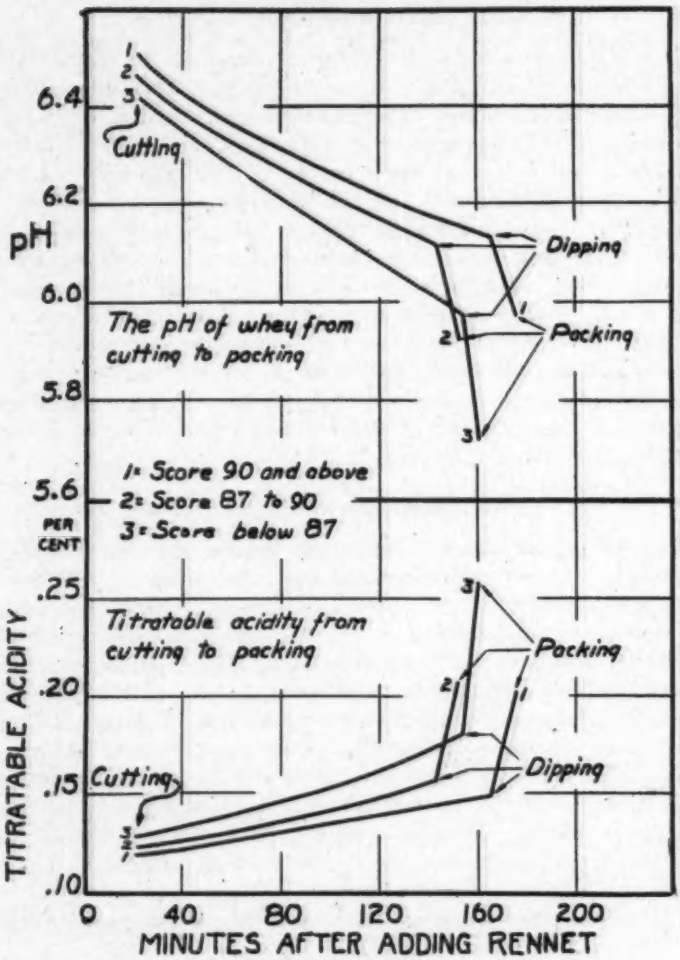

Figure 2

reason for this rise in pH but hardly within the scope of this paper to discuss it.

The average changes in acidity of the cheese in each group during curd-making are related to the time factor in figure 2. A significant difference between the three groups of cheese is illustrated in the angle of the break in each curve. The sharpest increase in acidity, after removing the whey, occurs in those lots of cheese in which the acidity develops most rapidly.

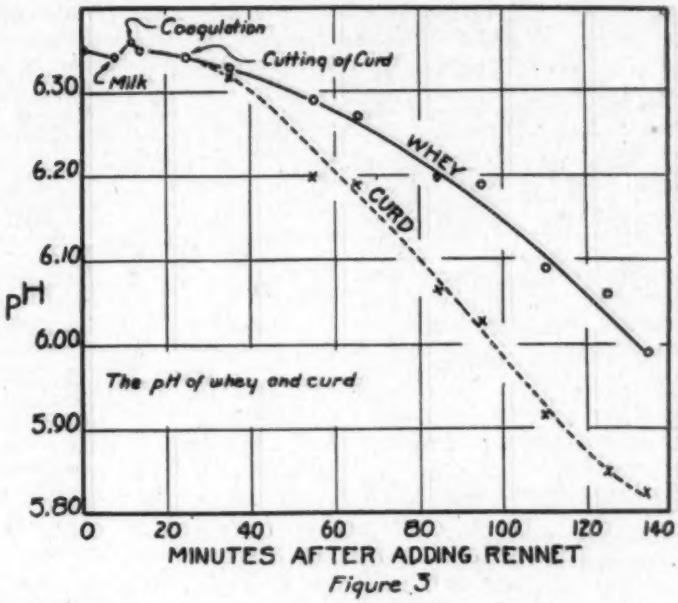

Figure 3

An explanation is suggested in the pH measurements of curd and whey from a batch of milk to which 5 per cent of starter was added. These measurements are plotted in figure 3.

It has been pointed out by Sammis, Suzuki, and Laabs (11) that the titratable acidity of the whey lags behind that of the curd. It is evident from figure 3 that pH measurements indicate the same trend. Other lots of cheese which showed less acid development in the making process before dipping were studied and it was found that as the rate of acid development decreases the difference between the pH of the whey and the curd decreases. It was also observed that after the bulk of the whey is removed from the curd that the pH of the small amount of whey which continues to drain from the curd approximates more closely, but not exactly, the pH of the curd. It was concluded that the changes in whey acidity between the intervals of dipping and packing, which are illustrated in figure 2, are indicative of the differences between the acidity of the whey and the curd which exist at the time of dipping and that these differences increase as the rate of acid development increases.

The relation between the acidity during curd-making and the quality of the cheese

The differences in acidity at the time of adding the rennet, as shown in tables 2 and 3, are not great enough to predict the quality of the cheese

which may result. This is true whether the acidity is measured as pH or by titration. At dipping and packing, however, the differences in acidity

TABLE 2

Average hydrogen ion concentration during the curd-making process

GROUP	I	II	III
Number of lots	14	25	15
Score of cheese	90 and over	87 to 90	below 87
	pH	*pH*	*pH*
Milk received*	6.56 ± 0.007	6.55 ± 0.006	6.55 ± 0.009
Rennet added*	6.47 ± 0.010	6.44 ± 0.007	6.40 ± 0.008
Cutting	6.51 ± 0.020	6.46 ± 0.007	6.42 ± 0.008
Heat on	6.43 ± 0.012	6.39 ± 0.007	6.37 ± 0.015
Heat off	6.35 ± 0.013	6.30 ± 0.009	6.26 ± 0.015
Whey removed	6.12 ± 0.016	6.12 ± 0.016	5.96 ± 0.031
Curd packed	5.97 ± 0.028	5.93 ± 0.021	5.74 ± 0.038
Curd milled‡	5.29 ± 0.027	5.29 ± 0.014	5.12 ± 0.023
Curd pressed‡	5.25 ± 0.019	5.20 ± 0.017	5.07 ± 0.020

* = measurements made on milk.
‡ = measurements made on curd.
All other measurements made on whey.

TABLE 3

Average titratable acidity during the curd-making process

GROUP	I	II	III
Number of lots	14	25	15
Score of cheese	90 and over	87 to 90	below 87
	per cent	*per cent*	*per cent*
Milk received	0.166 ± 0.0009	0.168 ± 0.0006	0.171 ± 0.0009
Rennet added	0.185 ± 0.0017	0.187 ± 0.0010	0.191 ± 0.0015
Cutting	0.122 ± 0.0015	0.125 ± 0.0007	0.129 ± 0.0014
Heat on	0.124 ± 0.0015	0.128 ± 0.0008	0.133 ± 0.0018
Heat off	0.131 ± 0.0020	0.135 ± 0.0010	0.142 ± 0.0025
Whey removed	0.151 ± 0.0031	0.157 ± 0.0024	0.181 ± 0.0040
Curd packed	0.201 ± 0.0056	0.210 ± 0.0054	0.259 ± 0.0105
Curd milled	0.556 ± 0.028	0.692 ± 0.028	0.781 ± 0.027
Curd pressed	No whey	No whey	No whey

between the three groups of cheese are considerably greater. This is more noticeable in figure 2 in which the acidity measurements are related to the time elapsing during the making operations. It is significant that the upper and lower halves of figure 2 are almost mirror images of each other. Regardless of the method of measurement, the highest scoring group of cheese is less acid at every stage of the making process, while the lowest scoring

group of cheese has the greatest acid development at every point of comparison.

It cannot be doubted from an examination of tables 2 and 3 and figure 2 that there is a definite relationship between the quality of the cheese and the acidity changes during the process of manufacture. A statistical study of the significance of the differences demonstrated by these pH and titratable acidity determinations indicates that the two methods of measurements are probably of the same relative value under the conditions of these experiments.

The relation between the pH and titratable acidity of the whey

In figure 4 the pH values of the individual samples of whey have been plotted against the corresponding titratable acidities. There is evidently a close correlation between these two methods of measuring acidity during the cheese-making process. Seventeen points beyond the range of the highest titratable acidity shown in figure 4, and 25 points which coincided with other observations between the pH limits 6.0 and 6.5, are not indicated, but they were used, however, in determining the slope of the original curve, of which figure 4 is a partial reproduction.

There is evidently a close correlation between these two methods of measuring acidity during the cheese-making process. It is not logical to believe, however, that the relationship which is implied by the curve in

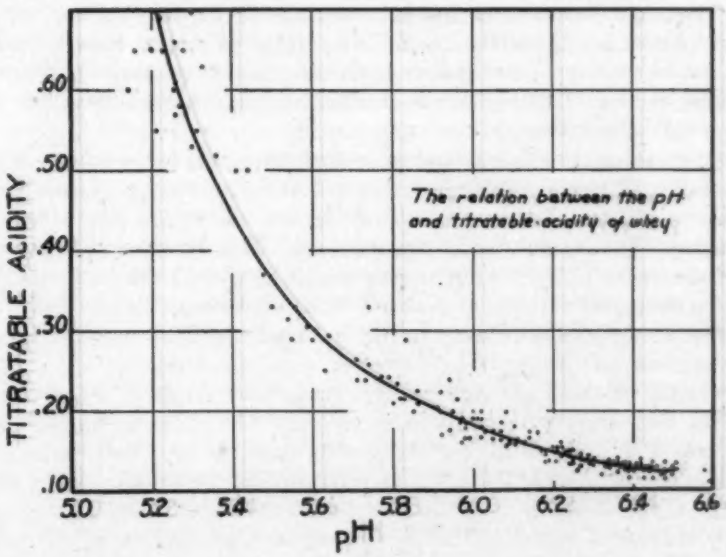

Figure 4

figure 4 would be constant under all conditions. Several factors might alter the slope of this curve if these experiments were duplicated. Three which should be mentioned are:

1. The types of organisms which dominated the acid development in the course of this study were probably characteristic of the local milk supply and of the starters which were used during the making experiments. Variations in these types might influence the identity of the products of fermentation and could logically affect the relation between the pH and the titratable acidity of the milk.

2. It has been demonstrated by Sharp and McInerney (1) that the initial acidity of fresh milk has a direct influence on the pH and titratable acidity measurements when that milk is allowed to sour. Cheese-making depends fundamentally on the development of acidity for the characteristic behavior of the curd. The initial acidity of the fresh milk undoubtedly influences the relation between these two methods of measuring acidity in the whey.

3. Studies of the buffer capacity of both individual and herd samples of milk (12, 13, 14 and 15) indicate the variable nature of this property. Obviously, variations in the buffer capacity would influence the nature of the curve shown in figure 4.

Acidity development in the cheese

The acidity measurements which were made during the ripening process of the cheese are summarized in table 4. These pH values show the same general trend in respect to quality as was exhibited in the cheese during the making process. The best cheese has the highest pH when fresh but the lowest pH after curing.

There are several fluctuations in acidity during the first week of curing which are difficult to explain. In general, however, there is a gradual increase in pH and increase in titratable acidity during the early stages of ripening. This is true for all three groups. It is interesting to observe that the cheese in the highest scoring group show the least change in pH during the period of these observations, while the cheese in the lowest scoring class show the greatest change in pH and finally actually attain a higher average than that developed by the better quality of cheese.

The pH values of the cheese in each group show a definite decrease during the first three or four days after making. One of the most significant features in this table is the regularity with which the best cheese maintains a pH above 5.0 during the entire ripening period. When the acidity of a cheese on the third day after it is made lays between 5.05 and 5.20, we have every reasonable assurance that it will not show acid defects at any later date.

TABLE 4

Average acidity during ripening

AGE OF CHEESE	GROUP I Score 90 and above		GROUP II Score 87 to 90		GROUP III Score below 87	
	pH	Titr. Ac.	pH	Titr. Ac.	pH	Titr. Ac.
		%		%		%
1 day	5.12	0.70	5.08	0.79	4.94	0.84
2 days	5.07	0.80	5.03	0.86	4.86	0.93
3 days	5.05	0.82	5.02	0.87	4.90	0.94
4 days	5.03	0.80	5.00	0.89	4.88	1.01
5 days	5.06	0.85	5.03	0.87	4.89	1.02
6 days	5.05	0.85	5.02	0.91	4.89	1.04
7 days	5.06	0.87	5.03	0.93	4.92	1.07
14 days	5.03	0.98	5.06	0.97	4.94	1.16
21 days	5.07	0.96	5.06	0.97	4.97	1.19
35 days	5.12	1.05	5.11	1.01	4.97	1.21
49 days	5.13	1.05	5.11	1.02	4.96	1.25
9 months	5.32	—	5.41	—	5.33	—
12 months	5.33	—	5.47	—	5.37	—
24 months	5.58	1.19	5.76	1.20	5.76	1.27
Difference between extremes	0.55	0.37	0.74	0.33	0.86	0.33

Unfortunately the titratable acidity measurements of the ripening cheese are not so dependable as are measurements of pH. The very nature of the method of analysis makes the results too variable. The degree of dispersion of the protein material which can be attained during the preparation of the sample for extraction varies decidedly in individual cheese during the first few weeks of curing. Those samples of cheese, which show the greatest tendency to dissolve in the warm water, carry through the filter the greatest amounts of protein material. The unsatisfactory relationship between the titratable acidity and pH measurements during the ripening of the cheese is more clearly shown in table 5, which presents the acidity measurements which were made on paired lots of cheese. Lots 82 and 83 were made from milk E, and lots 32 and 33 were made from milk K. The even numbered lots were made in a normal way to produce cheese of marketable quality. The other lots were treated in some manner to make high acid cheese. The results in table 5 illustrate the nature of the variations in the acidity measurements during the entire life of each cheese. They serve to emphasize the hopelessness of attempting to determine the quality of an unknown sample of cheese from a measurement of either pH or titratable acidity.

TABLE 5

The changes in pH and titratable acidity of individual cheese during making and ripening

MILK	E				K			
Cheese No.	82 (Score 90.8)		83 (Score 87.0)		32 (Score 87.3)		33 (Score 84.2)	
	pH	*Titr. ac.* %	*pH*	*Titr. ac.* %	*pH*	*Titr. ac.* %	*pH*	*Titr. ac.* %
Receiving	6.53	0.170	6.53	0.170	6.42	0.165	6.42	0.165
Setting	6.41	0.185	6.35	0.190	6.38	0.183	6.38	0.183
Cutting	6.45	0.120	6.36	0.135	6.48	0.125	6.48	0.123
Heat on	6.40	0.120	6.31	0.135	6.43	0.130	6.44	0.127
Heat off	6.34	0.130	6.24	0.145	6.34	0.133	6.36	0.135
Dipping	6.21	0.150	5.92	0.200	6.18	0.145	6.13	0.160
Packing	6.08	0.200	5.74	0.330	5.98	0.185	5.94	0.240
Milling	—	0.700	—	1.200	5.48	0.370	5.04	0.780
Pressing	5.38	—	5.04	—	5.45	—	5.02	—
1 day	5.09	0.70	4.92	0.86	5.28	0.68	4.93	0.85
2 days	4.97	0.94	4.84	1.15	5.20	0.70	4.77	0.90
3 days	4.99	0.94	4.83	1.08	5.19	0.68	4.81	1.04
4 days	4.99	0.94	4.93	1.19	5.12	0.61	4.81	1.01
5 days	5.07	1.04	4.97	1.15	5.12	0.72	4.88	1.08
6 days	5.05	0.97	4.91	1.22	5.18	0.79	4.83	1.04
7 days	5.06	1.04	4.93	1.24	5.11	0.72	4.82	0.94
14 days	5.12	1.03	4.95	1.24	5.23	0.72	4.92	1.04
21 days	5.20	1.04	4.95	1.15	5.18	0.65	4.90	1.19
35 days	5.16	1.11	5.01	1.26	5.32	0.83	5.01	1.22
49 days	5.12	1.06	5.01	1.21	5.19	0.76	4.94	1.33
70 days	5.16	1.10	5.04	1.28	—	—	—	—
9 months	5.28	—	5.16	—	5.45	—	5.42	—
12 months	5.30	—	5.29	—	5.42	—	5.45	—
24 months	5.58	1.12	5.69	1.22	5.72	1.17	6.01	1.15

CONCLUSIONS

1. Electrometric measurements of hydrogen ion concentration in the whey during the manfacture of cheddar cheese, apparently do not indicate significant changes in the acidity any more accurately than the ordinary titration test. In the ripening process, however, these measurements can be easily duplicated and although they are not necessarily indicative of the quality of the cheese they might be used to indicate its suitability for some specific purpose, such as processing.

2. The quality of cheddar cheese is closely related to the acid development during the cheese-making process. The hydrogen ion concentration and titratable acidity are high at each critical point when the quality of the cheese is inferior.

REFERENCES CITED

(1) Sharp, P. F., and McInerney, T. J. The relation of hydrogen ion concentration to the titratable acidity of milk. Jour. of Biol. Chem. 70: 729. 1926.

(2) Van Slyke, L. L., and Baker, J. C. Free lactic acid in sour milk. Jour. of Biol. Chem. 35: 147. 1918.

(3) Van Slyke, L. L., and Baker, J. C. Conditions causing variations in the reaction of freshly-drawn milk. Jour. of Biol. Chem. 40: 345. 1919.

(4) Rice, F. E., and Markley, A. L. The relation of natural acidity in milk to composition and physical properties. Journal of Dairy Science 7: 468. 1924.

(5) Knudsen, S. Über die Messung der Wasserstoffionenkonzentration des Käses mittels der Chinhydronelektrode. Zeit. Untersuch. Nahr. Genuss. 50: 300. 1925.

(6) Watson, Paul D. Use of quinhydrone electrode for following changes of pH in Swiss cheese. Jour. of Ind. Eng. Chem. 19: 1272. 1927.

(7) Watson, Paul D. Personal correspondence. 1930.

(8) Schulz, M. Über die Bedeutung der Wasserstoffionenkonzentration für die Beurteilung von Käse, insbes. Tilsiter Käse. Molkerei Zeitung 44: 527. 1930.

(9) Pasztor, S. Die Bestimmung der Wasserstoffionenkonzentration in Schmelzkäse mit Hilfe der Chinhydron-Methode. Molkerei Zeitung 44: 1831. 1930.

(10) Association of Official Agricultural Chemists. Official and Tentative methods of Analysis, p. 278, second edition, Washington, D. C. 1925.

(11) Sammis, J. L., Suzuki, S. K., and Laabs, F. W. Factors controlling the moisture content of cheese curds. Wisconsin Agricultural Station Research Bulletin 7, 1910.

(12) Holm, G. E., Webb, B. H., and Deysher, E. F. The heat coagulation of milk. 1. Variations in the compositions, heat stability and other tests of milks from four cows during the course of a lactation period. Journal of Dairy Science 15: 331. 1932.

(13) Buchanan, J. H., and Petersen, E. E. Buffers of milk and buffer value. Journal of Dairy Science 10: 224. 1927.

(14) Whittier, E. O. Buffer intensities of milk and milk constituents. 1. The buffer action of casein in milk. Jr. Biol. Chem. 83: 79. 1929.

(15) Holm, G. E., and Webb, B. H. Buffer capacities of various milks and proprietary infant foods. Am. Jr. Dis. Children 40: 260. 1930.

A STUDY OF FACTORS RELATED TO THE HARDENING OF ICE CREAM

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Recent studies conducted at this station, as well as elsewhere, have shown the importance of rapid freezing in the production of ice cream of smooth body. This applies not only to the ice formation that occurs in the ice cream freezer but that which takes place in the hardening room as well. Inasmuch as not more than half the water is usually frozen at the time the ice cream is drawn from the freezer, the hardening process becomes one of considerable importance. It was the purpose of this investigation to determine the relation of certain factors to the speed with which heat is removed from ice cream in a commercial type of hardening room.

PROCEDURE

Unflavored ice creams frozen in both batch and the Vogt continuous freezers were used. The hardening took place in a room 10 feet high, 8 feet wide, and 11 feet long, equipped with 1,150 feet of 1¼ inch expansion coils. Two adjacent rooms of this size were available.

All temperature readings were made by means of thermo-couples. This method is rapid, accurate to within one tenth of a degree, and adaptable for obtaining temperatures at any point in the product. The apparatus was placed on a table outside the hardening room which made it possible to check temperatures without entering the experimental area. The essential apparatus used was as follows:

1. Type K, Leeds and Northrup Potentiometer.
2. Type 2500, Leeds and Northrup Galvanometer.
3. Leeds and Northrup #30 D.C.C. Copper Thermo-couple wire.
4. Leeds and Northrup #30 D.C.C. Constantan Thermo-couple wire.

The thermo-couples were made by soft soldering the tips of the copper and constantan wire together. For protective purposes the tips were incased in slender paraffin filled glass tubes. The wires were coated with spar varnish so as to protect them against moisture. The thermo-couples were carefully calibrated against a Bureau of Standards thermometer. Readings were made every 30 minutes during the experimental period and unless otherwise specified all temperatures were taken at a central point in the container of ice cream.

FACTORS RELATED TO HEAT TRANSFER

When a difference in temperature exists between two adjacent bodies, there results a flow of heat toward the colder body. This transfer of heat

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may be due to either conduction, convection, radiation, or any combination of these three. An understanding of the characteristics of heat flow requires a general knowledge of these three types of heat propagation.

Conduction is a molecular transmission of heat, the material in question transmitting the heat from particle to particle of its own substance. If a flame is applied to one end of a copper rod, the heat passes toward the other end of the rod by conduction. The amount of heat transferred in this manner may be determined by use of the following equation:

$$Q = \frac{C\,A\,t_2 - t_1}{X}\,s$$

Q = quantity of heat in B.T.U.

C = coefficient of conduction.

A = area in square feet.

X = thickness in feet.

$t_2 - t_1$ = temperature difference between the two sections.

s = time in hours.

Convection denotes the transfer of heat energy within a liquid or gas by means of circulatory currents set up in the liquid or gas. The standard hot air heating system is an excellent example of this type of heat transmission. The rate of circulation depends principally upon the difference in density and viscosity of the hot and cold portions.

Radiation is the transmission of heat in space through an assumed medium commonly known as ether which is supposed to occupy all intermolecular space. The heat energy is supposedly converted into a wave motion of the ether and will thus even pass through clean dry air without a noticeable energy loss. All of the heat that the earth receives from the sun is in the form of radiant energy. The energy transmitted may be expressed as follows:

$H = K(T_1 4 - T_2 4)$.

H = quantity of heat in B.T.U. per square foot.

K = the radiation constant.

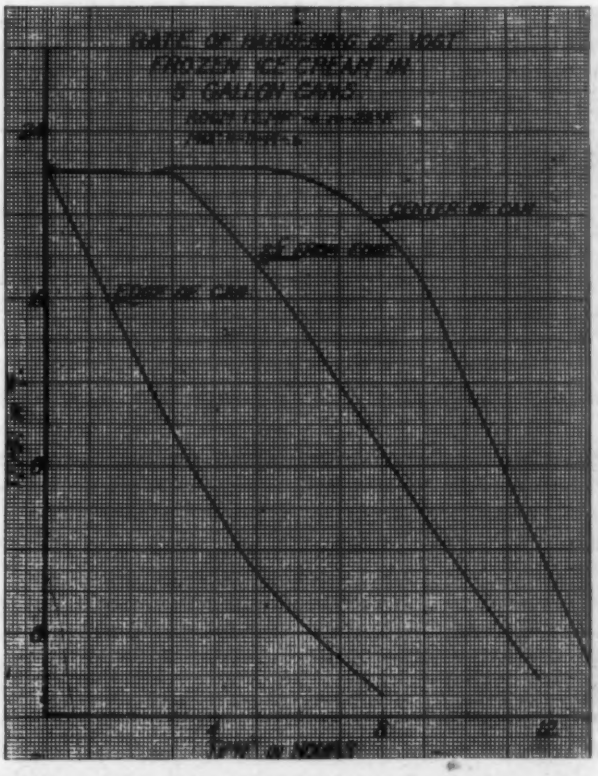
T_1 = absolute temperature of the radiating body.

T_2 = absolute temperature of the receiving body.

In the hardening room all of these three types of heat propagation are found but only conduction and convection are of major importance. The path of heat flow is naturally from the ice cream to the refrigerant which is usually confined ammonia. The heat which is derived from the latent and sensible heat of the ice cream will tend to flow through the cream and through the container walls much more rapidly than it can be removed from these walls by natural action. Therefore, the main problem is to accomplish the removal of heat from these walls as efficiently and rapidly as possible.

RATE OF TEMPERATURE CHANGE IN ICE CREAM WHILE HARDENING

To determine the rate of temperature change in a five-gallon metal can of ice cream during the hardening period the following experiment was performed. Thermo-couples were placed in the ice cream at the center of the can, 2¼ inches from the edge of the can and at the edge. All these junctions were 10 inches below the surface of the ice cream, which was one-half the depth of the can. Graph 1 shows the result of this study. In general,

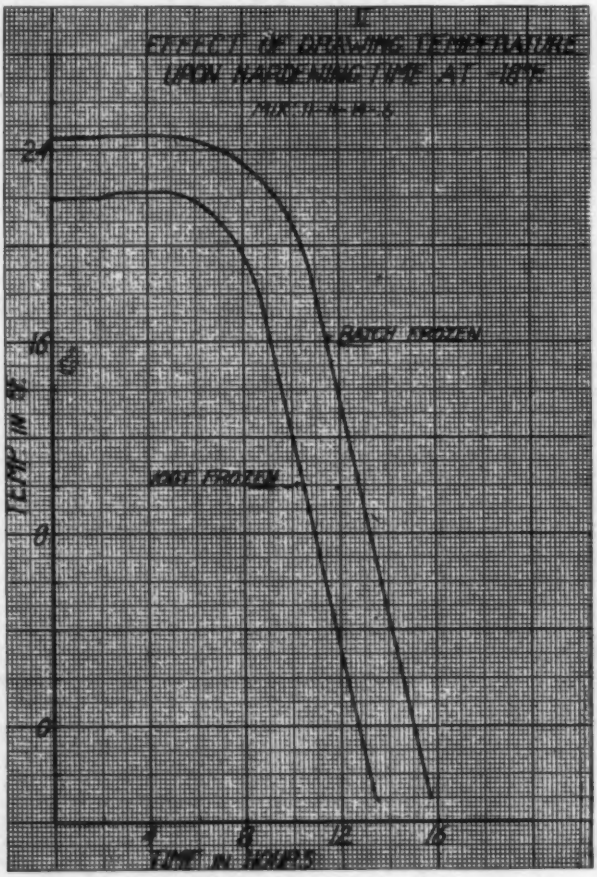


these data illustrate the insulating effect of the outer portion of the ice cream with regard to heat transfer from the center portion to the can walls. The temperature at the edge of the can started dropping immediately while that 2¼ inches from the edge did not start dropping appreciably until after three hours, while the portion in the center of the can remained at an almost constant temperature for approximately five hours. It will be noted that a slight increase (.2° F.) in the temperature of the

central portion occurred about three hours after the can had been placed in the hardening room and approximately two hours before the temperature curve started downward. This temperature rise was noted in practically all tests made in which the temperature was taken at the center of the can. The absence of a break in any of the temperature curves after they once started downward would indicate a cryohydric point in the ice cream is not reached within the temperature ranges studied.

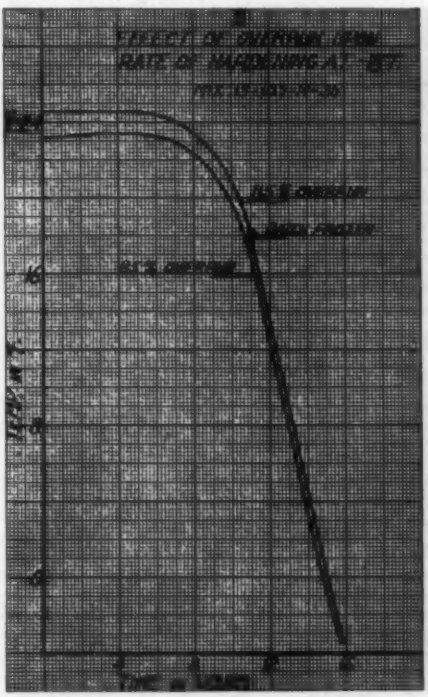
DRAWING TEMPERATURE

In general, the rate of hardening of ice cream depends upon the amount of heat to be removed and the rapidity with which the heat is conducted



away from the frozen product. The heat removed from the ice cream while in the hardening room consists of the sensible heat of the ice cream and the latent heat of the water that is frozen into ice during the hardening period. The percentage of unfrozen water is a function of the temperature of the ice cream. Therefore, the drawing temperature at the freezer is directly related to the percentage of water frozen and consequently to the time required to harden the ice cream.

By calorimetric methods it was determined that in the case of an ice cream mix containing 13 per cent fat, 11 per cent serum solids, 14.5 per cent cane sugar, 35 per cent of the water was frozen at 25° F., 52 per cent was frozen at 22° F., and approximately 82 per cent of the water was frozen at 0° F. It is evident, therefore, that the percentage of unfrozen water in the ice cream as it is drawn from the freezer has an important bearing upon the hardening time. That the drawing temperature is important is shown by graph 2 which shows the time required to harden ice cream in metal cans made from the same mix but frozen on different freezers. The batch frozen ice cream was drawn at a temperature of 24.5° F., whereas the Vogt frozen product was drawn at 22° F. The curves



run practically parallel but that of the Vogt ice cream reaches 0° F. a little over two hours sooner. In other words, a difference of 2.5° at the beginning of the hardening period meant a saving of approximately 16 per cent in the time required to reach 0° F. When ice creams were drawn from the two freezers at the same temperature identical hardening curves were secured.

OVERRUN

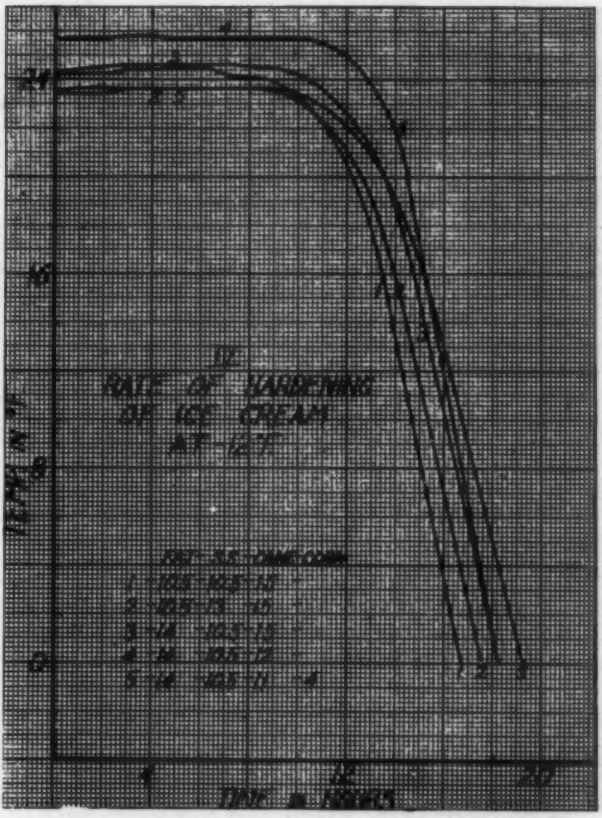
Graph 3 shows a comparison of the rate of hardening in metal cans of two five-gallon cans of the same ice cream differing only in overrun and consequently drawing temperature. It will be noted that although the 85 per cent overrun ice cream had a drawing temperature of 1.2° F. lower than that of the 115 per cent ice cream, the two reached zero at nearly the same time. Although the colder ice cream had a lower percentage of unfrozen water at the start, the greater amount of water that it contained per unit volume offset the effect of the temperature difference to a great extent. Since the hardening curves are much the same it may be concluded that the main effect of a change in overrun is a corresponding change in the number of heat units to be removed.

COMPOSITION OF ICE CREAM

Graph 4 shows the effect of variation in the milk solids and sugar content of the ice cream upon the rate of hardening. Varations in the soluble constituents of the ice cream resulted in differences in drawing temperatures due to the effect of these solids upon the freezing-point of the mix. It does not necessarily follow that ice creams drawn from the freezer at the same temperature will reach zero at the same time. This is shown by comparing the temperature curves of mixes 1 and 3, and 2 and 5. The rate of hardening increased as the initial freezing-point was raised as shown by comparing the temperature curves of mixes 3 and 4. Mix 5, which contained corn sugar and which had the lowest freezing-point had the slowest rate of hardening. This difference in the rate of hardening is directly traceable to the amount of unfrozen water. As the freezing-point is lowered the amount of water to be frozen between the temperature range of 20° and 0° F. increases which results in a decreased rate of fall in the ice cream temperature curve.

RAPID HARDENING

During the past few years rather extensive use has been made of electric fans specially made for hardening room operation. These fans have been found to shorten the hardening time which is an advantage not only from the standpoint of improved texture but also because of saving in time required to make the ice cream sufficiently hard to market. The fan used in this study was one supplied by the Meir Electric Company, of Indianapolis.

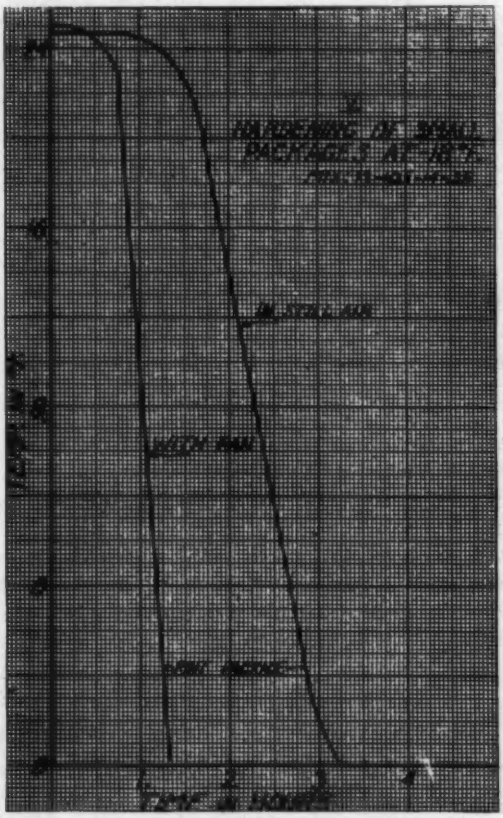


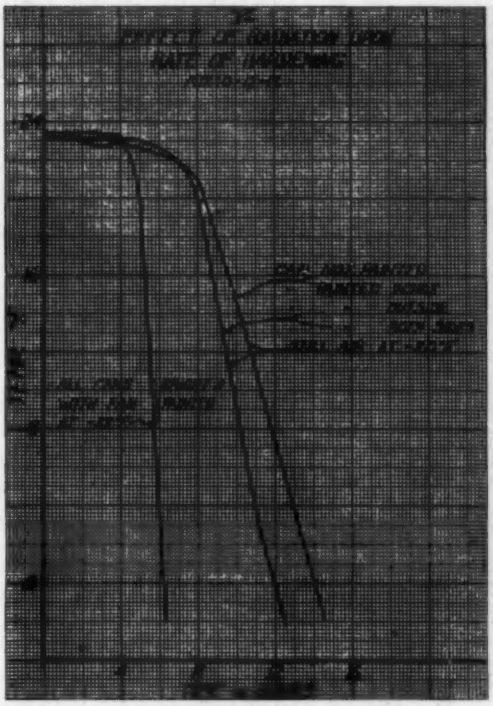
It was 24 inches in diameter and had an air velocity of 1,500 linear feet per minute. Hardening rooms identical in size and construction were used in comparing the rate of hardening ice cream with and without the use of an electric fan.

Heat passes from the ice cream container walls to the surrounding cold air by conduction and this air, becoming somewhat warmer, tends to rise thus carrying the heat away from the ice cream cans or packages. However, there is necessarily a thin film of stagnant air between the can wall and the moving air. As air is a very poor conductor of heat, this dead air film acts as an excellent insulator for the container. Therefore, the thickness of this air film to a great extent controls the rate of hardening. The conductivity coefficient of steel is 26.2 B.T.U. $\frac{\text{ft.}^2 \times {}^\circ\text{F.} \times \text{hr.}}{\text{ft.}}$, while that of air is approximately 0.0129. Using these figures it is easily determined

that if an air film of 0.01 of an inch thickness were to be placed by a steel wall of equal resistance to heat flow, this steel wall would be about 20 inches thick. Data from "Mark's Handbook" give the conductance of an air film (based upon quiet air and a 50° F. temperature differential) as 1.95 B.T.U. per square foot per °F. per hour. It is apparent, therefore, that the thickness or construction of the metal can wall is of minor importance in this type of heat transmission.

However, by the use of forced convection currents, such as those set up by a blast of air from a fan, the air film surrounding the container wall is partially swept away, thereby decreasing the hardening time. This saving in hardening time amounted to 50 per cent in the case of five-gallon cans. For smaller packages, an even greater reduction was made in the time required to harden to 0° F. Graphs 5 and 6 illustrate this point clearly.

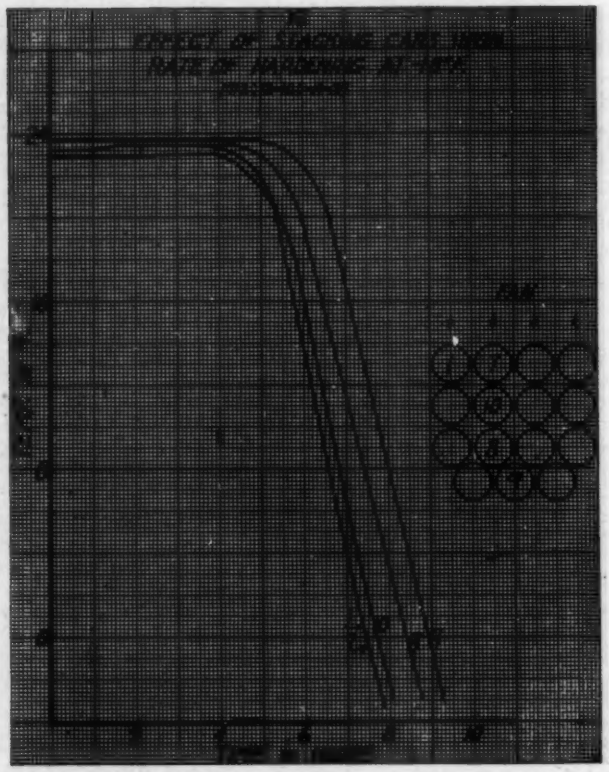




These comparative tests were made on identical samples placed in rooms of identical size and construction connected with the same compressor.

It might be added that the same type of resistance to heat transmission exists in the case of the heat passing from the air through the refrigerant piping. The air film around the pipes is also swept away to a great extent by the air blast thereby reducing the resistance. This action alone will enable the compressors to cool the room much more efficiently, due to the decreased temperature differential between the refrigerant and the circulating air. Observations made during hardening tests show that with a fan in operation, the same amount of heat can be removed per hour with a higher absolute back pressure. This will reduce power costs as well as increase the capacity of the compressors.

That the relative position of the fan and ice cream can is important is evidenced by graph 7 which shows the effect of stacked metal cans upon the rate of hardening. These cans were placed as close together as possible and a fan was placed about three feet in front of the first row of cans. The air velocity was decreased tremendously by the obstruction but hardening time under the condition of the experiment was not seriously affected. In the



cooling of stacked cans, the best efficiency will be obtained from the air blast by having the cans as much in the open as possible.

HEAT RADIATION FROM ICE CREAM CANS

So far no consideration has been given to radiation as a factor in the hardening room. From the definition of radiation, the cans may be expected to pass off some heat as radiant energy. Hardening tests upon five-gallon metal cans, standing alone in still air, have shown that ice cream cans painted on the outside with other than metallic paints will harden in about 85 per cent of the time required for ice cream in similar cans with bright surfaces. This is shown by graph 6. Painting the inside of the can was of no benefit. This is entirely in line with the theory of radiation, these data checking closely with reliable tests conducted upon steam and hot water radiators.[1] It is apparent therefore that ice cream cans painted

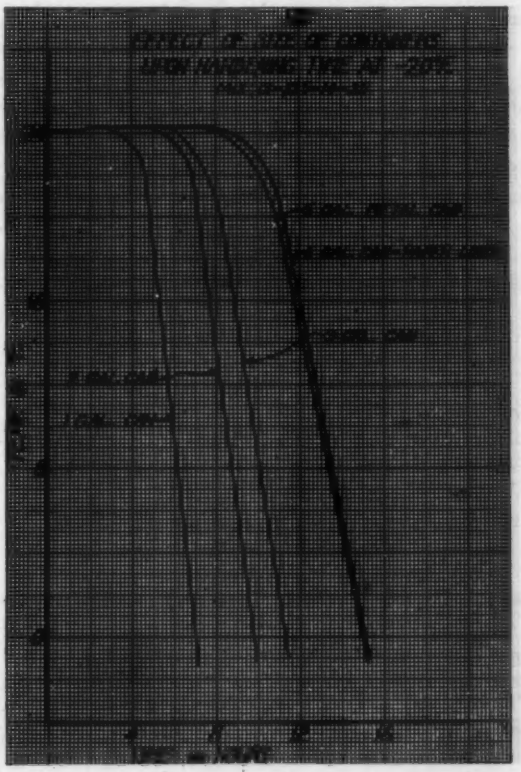
[1] Harding and Willard. Mechanical Equipment of Buildings. Vol. 1, p. 274.

on the exterior will be nearly perfect radiators while the opposite, will be true of unpainted cans.

However, this difference in hardening time due to radiation may not be attained in actual hardening room practise. If cans are stacked in groups, those that are enclosed upon all sides will receive as much energy as they send out, thereby reducing the effect of heat transmission in this manner to practically zero. Furthermore, if a fan is used, the total radiant energy emitted is greatly reduced. This is due to a decrease in hardening time and to a much lower surface temperature. The data below are from actual tests upon ice cream under practical conditions.

Temperatures – °F.

	Room	Can Wall	Center of 5-gal. Can
Natural convection	–17.4	– 5.6	19.3
Forced "	–18.3	–14.5	19.0



Substituting these known values in the formula for the radiant energy transmitted, $H = K(T_1^4 - T_2^4)$, in the case of natural convection (N) and forced convection (F) the following values are obtained—

$$H_N = K(454.2^4 - 442.4^4) = 10580 = 3.1$$
$$H_F = K(445.3^4 - 441.5^4) = 3370 = 1$$

It is evident, therefore, that under the conditions of the experiment the lower surface temperature noted under forced convection would reduce the amount of heat emitted by radiation at the can wall by about two-thirds.

As shown by the data in graph 6, in the case where forced convection was used there was no difference in the hardening time of the ice cream in the painted and unpainted cans. The unpainted can, however, contained ice cream which had a slightly higher initial temperature which probably accounts for the two curves coinciding in the lower temperature range, otherwise there would have been a slight difference in favor of the can painted black.

TYPE OF PACKAGE

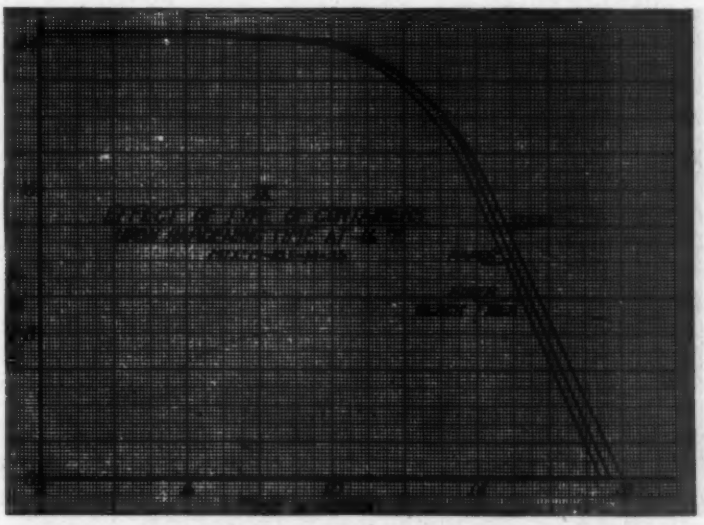
The hardening time for various size metal containers is shown in graph 8. The curve for five-gallon cans with paper liners has been closely checked and the maximum increase in hardening time was found to be under 2 per cent. Tests upon these containers and numerous packages have shown the hardening time to be a function of the cross sectional area and shape of the package. The data given below are for small paper packages hardened in still air at approximately – 18° F.

Shape of Package	Dimensions in Inches of Package	Paraffined	Initial Temp. of Ice Cream Degrees F.	Time to Harden to 0° F. in hrs.
Cylindrical	3½ dia. × 6½ long	Yes	24.3	4.65
	3½ " × 6½ "	No	24.3	4.60
	3½ " × 3 "	No	24.4	3.90
	5 " × 4½ "	Yes	24.0	5.50
Rectangular	2 × 3½ × 7½	Yes	24.6	3.95
	3 × 4½ × 5	Yes	24.1	4.35
	2½ × 3½ × 6½	Yes	24.8	4.60
Tapering	3½ – 2½ dia. × 4½	Yes	23.9	2.85
	4½ – 3½ " × 2½	Yes	24.1	3.25
	4½ – 3½ " × 3	Yes	24.1	3.50
	5½ – 4 " × 4	Yes	24.1	4.70

It it distinctly noticeable that the paraffining of paper packages did not decrease the rate of hardening. Paraffin undoubtedly will add to the re-

sistance to heat flow through the package wall but this addition is negligible when compared to the total resistance to the passage of heat.

In recent years, the industry has made extensive application of single service paper containers for marketing ice cream. Tests were made to compare the rate of hardening of ice cream in 20-quart containers of paper, fiber, and steel construction. The paper cans were laminated and were covered on the outside with sulphate paper and were lined on the inside with paraffin treated bond paper. The fiber cans were formed from wood fiber and were impregnated with water proofing material. The steel cans were of standard design with a bright tinned surface. It might be expected that the insulating effect of the paper would lengthen the hardening period, however, graph 9 shows that five-gallon paper or fiber cans standing alone



will harden faster than a five-gallon steel can under like conditions. A fiber can painted black showed no change in hardening time as compared to the unpainted can. This shows the fiber can to be a first-class radiator. However, in the case of stacked cans, where radiation is a negligible factor, the steel cans would probably show to better advantage in hardening time.

SUMMARY AND CONCLUSIONS

By means of thermo-couples accurate within 0.1° F., temperature changes occurring during the hardening of ice cream were studied. It was determined that in still air the temperature of the center portion of a five-gallon can of ice cream remained constant for about 5 hours. The

temperature then fell fairly rapidly, reaching zero after about 13 hours in a – 18° F. hardening room.

The drawing temperature of the ice cream is directly related to the percentage of water frozen and consequently the hardening time. A difference of 2½° in drawing temperature resulted in a saving of approximately 16 per cent (about 2½ hours) in the time required to reach zero.

Variations in overrun result in differences in drawing temperatures and amount of water per unit volume to be frozen. There was little difference in the hardening time of ice creams containing 85 and 115 per cent overrun due to the balancing effect of differences in initial temperature and amount of water present per unit volume.

As the freezing-point of the mix was raised the rate of hardening was increased. This was particularly true between 22° and 0° F. This was likely due to differences in the amount of water to be frozen in this temperature range, the high freezing point mixes having relatively more of the water frozen upon reaching 22° F.

By means of an electric fan placed in the hardening room the rate of hardening was increased approximately 100 per cent. This was thought to be due to the effect of the convection currents sweeping away the air film surrounding the ice cream container.

By painting the exterior surface of ice cream cans black it was possible to reduce the hardening time in still air about 15 per cent due to the greater heat radiation from the darkened surface. When hardened in the presence of forced convection currents the amount of heat transferred by radiation was greatly reduced due to a shorter hardening period and a lower temperature at the surface of the container.

Tests upon various types and sizes of ice cream packages show that the hardening time is dependent upon the area and shape of the package. Paraffining of paper packages did not decrease the rate of hardening.

Paper liners in five-gallon cans increased the time of hardening less than 2 per cent. Ice cream hardened faster in paper cans than in steel cans. Fiber cans conducted the heat from the ice cream more rapidly than either paper or steel cans. Painting fiber cans black did not alter the rate of heat transfer indicating the fiber surface to be a good radiator.

American Dairy Science Association Announcements

OFFICERS OF SECTIONS AND DIVISIONS

The newly elected officers of Sections and Divisions are given on the directory page as reported to the Editor.

EASTERN DIVISION MEETING

The twelfth annual meeting of the Eastern Division of the American Dairy Science Association was held September 17–19, 1933, at the Hotel Clinton, Springfield, Massachusetts. Attendance at the business meeting was 28 with chairman C. B. Bender, presiding. Due to conflicting meetings in Chicago the following day's program was poorly attended but 67 attended the banquet to hear Dr. J. G. Lipman speak on stabilizing the dairy industry.

The program included talks on artificially dried young grasses by J. A. Newlander, sanitary milk production by J. D. Brew, pasture grasses and clovers by R. C. Foley, succulence in the ration by G. C. White, vitamin A content of milk by W. C. Russell, the course in market milk by H. G. Lindquist, mastitis by J. G. Hucker, and mastitis and soft curd milk by R. C. Welch and F. J. Doan.

R. W. Smith, Jr., reported that in the Dairy Products Judging Contest Vermont was first, Massachusetts second, and Connecticut was third. Five teams competed. In the Dairy Cattle Judging Contest, A. R. Merrill reported that Maryland was first, Rutgers was second, and Cornell was third. In the judging of General Livestock, E. N. Boland reported that Pennsylvania was first, Massachusetts was second, and New Hampshire was third.

R. E. JOHNSON, *Secretary.*